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RESEARCH ARTICLE

TISSUE CULTURE STUDIES ON *SCOPARIA DULCIS* L.

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Abstract

The present study is aimed at the plant regeneration from internode, leaf, node and shoot tip cultures of *Scoparia dulcis* L. (Scrophulariaceae), a branched herb with high potential in traditional and folk medicine. The effects of the different media with MS composition supplemented or not with varying concentrations of plant growth regulators on the cultures of *Scoparia dulcis* as well as the variation in response of four different explants were studied. The results are varying in different media supplemented with 2,4-D, IBA, BA and also in basal medium. Maximum response was obtained on supplementing the MS medium with IBA.

Introduction:-

The process of plant regeneration from isolated explants starts with dedifferentiation, redifferentiaon and organization into meristematic centres, leading to the formation of unipolar structures i.e. organogenesis, or bipolar structures called somatic embryogenesis. *Invitro* culture is rooted on the concept of cellular totipotency by Haberlandt (1902) and was uniequivocally demonstrated for the first time by Steward et al.(1958) who discovered somatic embryos from carrot cells. Tissue culture which broadly embraces cell, tisuue and organ culture through *in vitro* conditions (Debergh and Read, 1991) can be employed for large scale propagation of disease free clones and gene pool conservation of elite superior varieties. Addition of plant hormones becomes essential for shoot induction and multiplication. Plant tissue culture techniques have been increasingly employed with great success in propagating both difficult and easily propagated taxa such as *Capsicum annuum* (Sobhakumari and Lalithakumari 2003), *Ficus benjamina* (Rzepka- Plevnes and Kurek, 2001), *Dracaena deremensis*(Chua et al., 1981) etc.

Scoparia dulcis (L.) (Scrophulariaceae), commonly known as sweet-broom, bitter broom or rice weed is a branched herb with wiry stems, up to 1m tall. The species is widely distributed in tropical and subtropical regions of South America and Asia. The leaves are opposite or whorled in threes, narrowly elliptic, subsessile, 3-4cm long, 1-1.5 cm wide and distantly serrulate or serrate. The small white flowers are pedicellate and axillary, with 4- lobed calyx; 5mm wide corolla which is hairy within, greenish stamens, green ovary and subglobose capsule. Propagation is through seeds. The plant is used in folk medicine to treat respiratory, gastric and hepatic disturbances and as anti-inflammatory. It is also used in India for the treatment of diabetes and hypertension, ear ache, head ache, tooth ache, anemia, antiseptic, astringent, antidote against snakebite, conjunctivitis, cough, diarrhoea, fever, gonorrhoea, jaundice, disease of the kidney, piles rash, retinitis, sores, sore throat, tumors, venereal diseases etc.(Gonzales-Torres, 1986; Kawasaki et al. 1987). Increasing demand of a taxon in herbal medicine, calls for application of non-conventional methods of propagation for commercial utilization and conservation. Such high demands can be satisfied only via alternative crop improvement programs utilizing *in vitro* methods, which may help in large-scale

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clonal propagation of the plant. The present study aims at developing an efficient protocol for the rapid propagation of *Scoparia dulcis* through internode, leaf, node and shoot tip cultures, to study the effects of basal medium and various concentrations of plant growth regulators on the cultures and evaluate the responses elicited by these hormones from the four different explants.

Materials And Methods:-

The plant material selected for the study viz. *Scoparia dulcis* L. , was grown in pots in the department garden of Sree Narayana College, Kollam, Kerala, South India. Various explants like internodal, leaf, nodal and shoot tip segments were collected from the mother plant, surface sterilized with appropriate chemicals including 0.1% $HgCl_2$ solution and inoculated into different media with MS composition (Murashige and Skoog, 1962), supplemented or not with varying concentrations of plant growth regulators such as 2,4- Dichlorophenoxyaceticacid,(2,4- D), Indole Butyric acid(IBA) and Benzyl Adenine (BA). The inoculated medium containing explants were incubated at 25± 1°C, 50-90 Relative Humidity and 2000 lux light intensity in the culture room, on a12/12 h photoperiod. Regeneration and callusing responses were recorded regularly and photoghraphs taken. Each hormone was prepared in five different concentrations (0.1mg/l, 0.5mg/l, 1mg/l, 3mg/l and 5mg/l and the effect of each concentration on all the four explants were studied. The effects of the different media on the cultures of *Scoparia dulcis* as well as the variation in the responses of the four different explants to these treatments were studied. The experiments were conducted in ten replicates, to minimize error.

Results and Discussion:-

The observations from the study are furnished in the tables given below :

Table I:-Effects of basal medium and various concentrations of growth hormones on callus induction in *Scoparia dulcis* L.

Basal medium/ growth hormone	Concentration (mg/l)	Callus induction (+/-)	Nature of callus	Frequency of callus induction(%)
BM	-	-	-	-
2,4-D	0.1	+	Creamy brown/ dark brown/creamy black spongy	100
	0.5	+	Creamy yellow/creamy black spongy	100
	1	+	Creamy spongy	100
	3	+	Creamy brown spongy	100
	5	+	Creamy brown spongy	100
IBA	0.1	+	creamy yellow/brown spongy	20
	0.5	+	Yellow spongy turning brown or white	70
	1	+	Creamy friable/ creamy yellow spongy	80
	3	+	Creamy yellow spongy turning brown	80
	5	+	Creamy yellow spongy /nodular	85
BA	0.1	-	-	-
	0.5	-	-	-
	1	-	-	-
	3	-	-	-
	5	-	-	-

*Mean ± SE

Table II:-Effects of Basal medium and various Growth Hormones on rhizogenesis in *in vitro* raised shoots of *Scoparia dulcis*

Basal medium/ growth hormone	Concentration (mg/l)	% of shoots showing rooting	No. of roots per shoot	Length of longest root*(cm)	Root dimorphism (+/-)	Nature of roots produced
BM	-	20	1.3±0.152	2.6±0.163	-	Creamy white hairy
2,4-D	0.1	-	-	-	-	-
	0.5	-	-	-	-	-

	1	-	-	-	-	-
	3	-	-	-	-	-
	5	-	-	-	-	-
IBA	0.1	100	22.1±1.77	6.7±1.63	+	One green long thick main root and short white tufted lateral roots
	0.5	50	2.8±0.327	2.2±0.250	-	Short branched white hairy ,often entangled
	1	30	4.7±0.367	2.7±0.201	-	Light green/ white hairy roots, moderately long
	3	50	5.0±1.10	1.6±0.139	-	Slender white hairy/ short thick very hairy roots
	5	50	1.4±0.267	1.3±0.088	-	Short thick whitish yellow hairy with bulged tips
BA	0.1	-	-	-	-	-
	0.5	-	-	-	-	-
	1	-	-	-	-	-
	3	-	-	-	-	-
	5	-	-	-	-	-

*Mean ± SE

Table III:-Effects of Basal medium and various Growth Hormones on shoot regeneration in *Scoparia dulcis*

Basal medium/ growth hormone	Concentration (mg/l)	% of cultures showing shoot regeneration	No. of shoots regenerated per culture*	No. of elongated shoot buds per culture*
BM	-	75	4.6±0.163	1.8±0.133
2,4-D	0.1	-	-	-
	0.5	-	-	-
	1	-	-	-
	3	-	-	-
	5	-	-	-
IBA	0.1	75	6.4±0.221	1.6±0.221
	0.5	-	-	-
	1	-	-	-
	3	-	-	-
	5	-	-	-
BA	0.1	40	33.7±4.14	-
	0.5	100	128.1±9.65	-
	1	60	103±12.00	-
	3	-	-	-
	5	-	-	-

*Mean ± SE

Table IV:-Effects of Basal medium and various Growth Hormones on shoot elongation in *Scoparia dulcis*

Basal medium/ growth hormone	Concentration (mg/l)	Height of the shoot*(cm)	No. of nodes per shoot*	Length of internode*(cm)
BM	-	6.0±0.472	3.9±0.278	1.5±0.132
2,4-D	0.1	-	-	-
	0.5	-	-	-
	1	-	-	-
	3	-	-	-
	5	-	-	-
IBA	0.1	11.4±0.236	6.2±0.443	2.5±0.085

	0.5	10.2±0.318	6.3±0.367	1.9±0.060
	1	5.8±0.250	4.8±0.250	1.1±0.073
	3	2.2±0.490	2.5±0.342	1.1±0.090
	5	2.9±0.224	3.8±0.250	1.3±0.072
BA	0.1	2.4±0.125	2.5±0.167	-
	0.5	-	-	-
	1	-	-	-
	3	-	-	-
	5	-	-	-

*Mean ± SE

Table V:-Response of the different explants of *Scoparia dulcis* to Basal medium

Type of explant	Callus induction		Shoot regeneration		Shoot elongation		Rhizogenesis	
	%	Nature	%	No. of shoots/ culture*	%	Length of longest shoot*(cm)	%	No. of shoots per shoot*
Internode	-	-	30	4.4±0.163	30	2.0±0.258	50	0.8±0.133
Leaf	-	-	-	-	-	-	-	-
Node	-	-	-	-	100	6.4±0.401	20	1.2±0.250
Shoot tip	-	-	-	-	100	6.7±0.261	30	1.1±0.234

*Mean ± SE

Table VI:-Response of the different explants of *Scoparia dulcis* to various concentrations of 2,4-D

Conc. (mg/l)	Type of explant	Callus induction		Shoot regeneration		Shoot elongation		Rhizogenesis	
		%	Nature	%	No. of shoots/ culture*	%	Length of longest shoot*(cm)	%	No. of shoots per shoot*
0.1	Internode	100	Creamy spongy	-	-	-	-		--
	Leaf	100	Cream brown	-	-	-	-	-	-
	Node	100	Cream brown	-	-	-	-	-	-
	Shoot tip	100	Cream brown/ black	-	-	-	-	-	-
0.5	Internode	100	Creamy yellow	-	-	-	-		--
	Leaf	100	Creamy brown	-	-	-	-	-	-
	Node	100	Creamy black	-	-	-	-	-	-
	Shoot tip	100	Creamy bown	-	-	-	-	-	-
1	Internode	100	Creamy spongy	-	-	-	-		--
	Leaf	100	Creamy brown	-	-	-	-	-	-
	Node	100	Creamy brown	-	-	-	-	-	-
	Shoot tip	100	Creamy spongy	-	-	-	-	-	-
3	Internode	100	Creamy spongy	-	-	-	-		--
	Leaf	100	Creamy black	-	-	-	-	-	-
	Node	100	Creamy brown	-	-	-	-	-	-
	Shoot tip	100	Creamy brown	-	-	-	-	-	-
5	Internode	100	Creamy spongy	-	-	-	-		--
	Leaf	100	Creamy brown	-	-	-	-	-	-
	Node	100	Creamy brown	-	-	-	-	-	-
	Shoot tip	100	Creamy brown	-	-	-	-	-	-

*Mean ± SE

Table VII:-Response of the different explants of *Scoparia dulcis* to various concentrations of IBA

Conc. (mg/l)	Type of explant	Callus induction	Shoot regeneration	Shoot elongation	Rhizogenesis

		%	Nature	%	No. of shoots/ culture*	%	Length of longest shoot*(cm)	%	No. of shoots per shoot*
0.1	Internode	100	Creamy yellow	100	1.6±0.221	100	9.9±0.194	100	12.6±0.476
	Leaf	-	-	80	7.9±0.407	100	3.3±0.367	100	10.6±0.499
	Node	-	-	50	3.5±0.543	100	12.2±0.291	100	7.3±0.598
	Shoot tip	-	-	60	3.5±0.402	100	10.3±1.03	10	4.5±0.342
0.5	Internode	100	Creamy spongy	-	-	-	-		--
	Leaf	90	Creamy spongy	-	-	-	-	-	-
	Node	-	-	-	-	100	10.9±0.208	100	2.9±0.277
	Shoot tip	50	Creamy yellowish white	-	-	50	9.9±0.174	50	2.3±0.214
1	Internode	80	Creamy friable	-	-	-	-		--
	Leaf	100	Creamy yellow	-	-	-	-	100	5.8±0.250
	Node	40	Creamy spongy	-	-	40	5.7±0.213	40	3.9±0.234
	Shoot tip	90	Creamy yellow	-	-	-	-	-	-
3	Internode	80	Creamy brown	-	-	-	-	100	2.1±0.180
	Leaf	100	Creamy brown	-	-	-	-	100	8.0±0.395
	Node	60	Creamy yellow	-	-	80	2.5±0.402	30	1.6±0.163
	Shoot tip	80	Creamy brown	-	-	-	-	40	2.3±0.301
5	Internode	100	Creamy brown	-	-	-	-	100	2.3±0.261
	Leaf	80	Creamy brown	-	-	-	-	100	1.7±0.301
	Node	60	Creamy brown	-	-	40	2.4±0.145	-	-
	Shoot tip	30	Creamy nodular	-	-	-	-	-	-

*Mean ± SE

Table VIII:-Response of the different explants of *Scoparia dulcis* to various concentrations of BA

Conc (mg/l)	Type of explant	Callus induction		Shoot regeneration		Shoot elongation		Rhizogenesis	
		%	Nature	%	No. of shoots/ culture*	%	Length of longest shoot*(cm)	%	No. of shoots per shoot*
0.1	Internode	-	-	70	17.4±0.670	-	-	-	-
	Leaf	-	-	60	41.3±2.30	-	-	-	-
	Node	-	-	30	27.8±0.728	-	-	-	-
	Shoot tip	-	-	-	-	20	2.2±0.082	-	-
0.5	Internode	-	-	100	2.5.8±1.23	-	-		--
	Leaf	-	-	100	130±6.99	-	-	-	-
	Node	-	-	100	154.5±1.17	-	-	-	-
	Shoot tip	-	-	100	134.4±3.60	-	-	-	-
1	Internode	-	-	30	17.9±0.7.7	-	-		--
	Leaf	-	-	70	61.3±2.26	-	-	-	-
	Node	-	-	40	24.4±1.89	-	-	-	-
	Shoot tip	-	-	100	120.5±3.21	-	-	-	-
3	Internode	-	-	-	-	40	Slight cream callusing	-	-
	Leaf	-	-	-	-	100	Crowding of leaves	-	-
	Node	-	-	-	-	100	Compact shooting	-	-
	Shoot tip	-	-	-	-	-	-	-	-
5	Internode	-	-	-	-	-	-		--
	Leaf	-	-	-	-	-	-	-	-
	Node	-	-	-	-	70	Short axillary shoot	-	-
	Shoot tip	-	-	-	-	60	Compact shooting	-	-

*Mean ± SE

Table IX:-Response of the different explants of *Scoparia dulcis* to various concentrations of 2,4- D and BA

Type of explant	Callus induction		Shoot regeneration		Shoot elongation		Rhizogenesis	
0.5mg/l BAP & 0.5mg/l 2,4-D								
	%	Nature	%	No. of shoots/ culture*	%	Length of longest shoot*(cm)	%	No. of shoots per shoot*
Internode	100	creamy spongy	-	-	-	-	-	-
Leaf	100	Creamy green	-	-	-	-	-	-
Node	-	-	-	-	100	2.19±0.207	-	-
Shoot tip	70	Greenish yellow	-	-	20		-	-
0.5mg/l BAP & 1mg/l 2,4-D								
Internode	100	Creamy yellow	-	-	-	-		--
Leaf	100	Creamy yellow	-	-	-	-	-	-
Node	100	Creamy yellow	-	-	-	-	-	-
Shoot tip	100	Creamy yellow	-	-	-	-	-	-
1 mg/l BAP & 0.5mg/l 2,4-D								
Internode	100	Creamy yellow	-	-	-	-		--
Leaf	100	Creamy green	-	-	-	-	-	-
Node	100	Creamy spongy/friable	-	-	-	-	-	-
Shoot tip	100	Creamy spongy	-	-	-	-	-	-
1 mg/l BAP & 1mg/l 2,4-D								
Internode	100	yellow green	-	-	-	-	-	-
Leaf	100	Creamy green	-	-	-	-	-	-
Node	100	Yellow green	-	-	-	-	-	-
Shoot tip	100	Creamy green	-	-	-	-	-	-

*Mean ± SE

A. Basal Medium

Basal medium did not induce calli. Moderate rhizogenesis was observed. Among the four explants studied, the internodal segments responded the most (50%) producing 0.8±0.133 roots per shoots. Direct shoot elongation and in vitro flowering were observed in 30 days. The leaf segments showed shoot elongation in basal medium. The nodal and shoot tip segments showed the response of producing 6.4±0.401cm and 6.7±0.261cmlong shoots respectively.

B. 2,4-D in MS Medium

MS Medium supplemented with 2,4-D resulted in callus induction alone. Confirms the earlier observations of 2,4-D inhibiting the organogenic capacity of calli. The calli produced were mostly creamy spongy or creamy brown, rarely creamy black. The internodal ends showed creamy callusing in 0.1mg /l2,4-D. The leaf explant exhibited creamy callusing from the margin and midrib in 0.5mg/l 2,4-D and heavy creamy brown or black callusing was observed in 3mg/l 2,4-D. The nodal explants showed basal creamy compact callusing and shoot tip exhibited basal creamy black callusing.

C. IBA in MS Medium

Maximum response was obtained on supplementing the MS medium with IBA. Callus induction, rhizogenesis with dimorphic roots, shoot regeneration and shoot elongation and in vitro flowering observed. The calli induced were mostly creamy yellow or brown spongy or cream friable. The callus induction is increased gradually from 0.1mg/l to 5mg/l concentration of IBA in the MS medium. Maximum rhizogenesis was observed at 0.1mg/l IBA with dimorphic roots (22.1±1.77). In higher concentrations of IBA (0.5, 1 and 3mg/l) the roots were often entangled, light green or white hairy and moderately long with buldged tips were observed. The roots produced in 5mg/l were the shortest (1.3±0.088cm) and least in number (1.4±0.267). Shoot regeneration was observed only in 0.1 mg/l concentration of IBA with 6.4±0.778 shoots per culture. Maximum shoot elongation (11.4±0.236) with 6.2±0.443 nodes on an average, separated by 2.5±0.0825cm long internodes, was obtained in 0.1 mg/l IBA. The shoot height, nodal number and internodal length decreased at higher concentrations of IBA from 0.5 mg/l to 5 mg/l.

D. BA in MS Medium

Callus induction and rhizogenesis were absent in all concentrations of BA. Maximum shoot induction (100%) was observed in 0.5 mg/l BA with the nodal segments producing as high as 154.5 ± 1.17 shoot buds per culture. The nodal segments responded the most producing as high as 154.5±1.17shoot buds. The internodal segments yielded

the lowest number of shoot buds (25.8±1.23). The number of shoot buds regenerated from leaf and shoot tip explants were 130±6.99 and 134.4±3.60 respectively. All the nodal and shoot tip segments exposed to 3mg/l BA in the medium, showed produced short, compact shoots with the leaves crowded together at the top. It is suggested that the addition of auxins or GA3 to the medium along with BA might circumvent the dwarfing effected by BA

E. 2,4-D & BA in MS Medium

Addition of BA along with 2,4-D in the MS medium resulted in 100% callus induction. But shoot regeneration, elongation and rhizogenesis were lacking. The internodal and leaf explants exhibited 100% callusing while 70% of shoot tip segments produced greenish yellow calli. The nodal segments alone failed to respond. A combination of 0.5mg/l BA and 0.5 mg/l 2,4-D in the medium resulted in 100% creamy callusing from all expalnts. Medium with a combination of 1mg/l BA and 0.5mg/l 2,4-D produced creamy yellow spongy callusing of entire internodal segments. Addition of 1mg/l BA and 1mg/l2,4-D in the medium resulted in the formation of yellow or creamy green calli. Although the presence of 2,4-D in the medium induced only calli in all treatments, adequate proliferation of the calli did not follow, possibly due to the antagonistic effect of BA.

A good response was shown by internodal, nodal and shoot tip explants in hormone free basal media in the present study, similar observations were made by Escandon et al. (2005). The leaf explants in 0.1 mg/l 2,4-D displayed dark brown spongy callusing. Indira et al (2002) found that explants from *Myristica fragrance* cultured in MS medium with 2,4-D produced whitish granular callus, while those from immature leaves produced soft , translucent, friable callus with embryo like structures. Bonfill et al (2002) have observed that IBA increases the organogenic capacity of the *Panax ginseng* calli, and induced formation of high number of buds and roots. The present study also supports the observation. Escandon et al. (2005) studied the nodal segment multiplication of five *Scoparia* species including *S. dulcis*, in MS medium supplemented with 0.25 mg/l BAP and found that except for *S. hasleriana*, the multiplication rate of the other species ranged between10 and 12 shoots per explants. But in the present study nodal segments responded the most producing as high as 154.5±1.17 shoots per culture. Addition of BA along with 2,4-D in the MS medium resulted in 100% callus induction in all the four combinations studied. Stimulation of callus in shoot apex *in vitro* cultures of *Rauwolfia tetraphylla* on MS basal medium supplemented with NAA and BA only or both with IAA and NAA combined with BA was reported by Ghosh and Banerjee (2003).

Conclusions:-

The present study focuses on the plant regeneration of from internode, leaf, node and shoot tip ~~of~~ cultures of *Scoparia dulcis* L. in MS media composition and varying concentration of plant growth regulators. Basal medium did not induce calli. MS medium supplemented with 2,4-D resulted callus induction alone. Maximum response was obtained on supplementing the MS medium with IBA. Callus induction, rhizogenesis, shoot regeneration, maximum shoot elongation, *in vitro* flowering were observed in cultures of four explants in varying concentrations of IBA. Callus induction and rhizogenesis were absent in all concentrations of BA. Shoot regeneration was observed only in the lower concentrations of BA (0.1-1mg/l).The combinations of BA and 2,4-D induced callus formation. The absence of shoot regeneration, shoot elongation and rhizogenesis in all treatments could be attributed to the antagonistic effect of auxin, inhibiting shoot bud formation, as has been reported earlier.

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Fig 1
Fig 2
Fig 3
Fig 4
Fig 5
Fig 6
Fig 7
Fig 8
Fig 9
Fig 10
Fig 11
Fig 12
Fig 13
Fig 14
Fig 15
Fig 16
Fig 17
Fig 18
Fig 19
Fig 20

Figs 1 -2 : shoot regeneration in BM; **Fig.3** – creamy callus**; Fig.4** – creamy brown callus (3 mg/l 2,4-D) ; **Fig.5 –** brown spongy callus with shoot regeneration (0.1 mg/l 2,4-D) ; **Fig.6** – cream spongy callus; **Fig.7** –shoot regeneration (0.1 mg/l BA) ; **Fig.8** – brown callus (0.5 mg/l IBA) ; **Fig.9** – rhizogenesis & shoot elongation (0.5 mg/l IBA) ; **Fig.10** – creamy yellow callus (5 mg/l IBA) ; **Fig.11** – callus, shoot elongation & rhizogenesis (1 mg/l IBA) ; **Fig.12** – multiple shoot regeneration (0.5 mg/l BA) ; **Fig.13** – proliferation of shoot tip (0.1 mg/l BA) ; **Fig.14** – multiple shoot proliferation (0.5 mg/l BA) ; **Fig.15** – creamy spongy callus (0.5 mg/l BA + 0.5 mg/l 2,4-D) ; **Fig.16** –spongy yellowish green callus (0.5 mg/l BA + 0.5 mg/l 2,4-D) ; **Fig.17** – creamy green basal callus (1 mg/l BA + 1 mg/l 2,4-D) ; **Fig.18** –basal spongy yellow callus (1 mg/l BA + 0.5 mg/l 2,4-D) ; **Fig.19** – rooted explants in liquid medium ; **Fig. 20** – callus for long term storage.

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