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Effect of Rhustox on Micropropagation of *Scoparia dulcis* L. through Leaf Explants Culture

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Authors' contributions

This work was carried out in collaboration between both authors. Author SZ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript and author MPT managed the analyses of the study. Authors SZ and MPT managed the literature searches. Both the authors read and approved the final manuscript.

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ABSTRACT

Aim: To investigate the effect of Rhus toxicodendron (30CH) along with different compositions of phytohormones (Auxin and Cytokinin) on the basis of growth and multiplication of explants under optimum temperature under *in-vitro* conditions.
Study Design: To establish and design the standard protocol for the *in-vitro* propagation through leaf explant of *Scoparia dulcis* under stress of phytohormones and homeopathic medicine Rhus toxicodendron (30CH).
Place and Duration of Study: The plant materials were procured from the Herbal Botanical Garden Patna Science College, Department of Botany, Patna University, Patna, Bihar. The experimental part was carried out in Plant Tissue Culture Laboratory, between December 2017 to August 2018 in Department of Botany P.U. Patna.
Methodlogy: The sterilized leaf explants were inoculated into MS media fortified with different phytohormones (Auxin and Cytokinin) and Rhus tox(30CH) under aseptic environmental conditions for the growth and development of callus, embryoids etc.
Result: The explants in MS medium supplemented with auxins phytohormones and Rhus tox(30CH) exhibited that IAA (0.10 to 2.0 mg/l) and BAP (0.10 to 2.5 mg/l) induces green and

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compact calli. Whereas at 0.30mg/l of IAA and 0.50 mg/l BAP induced brown friable calli. 2,4-D (1.5 mg/l) and Kinetin (1.5-6.5mg/l) concentrations induced brown and friable calli. Rhus tox(30CH) (100 µl/100 ml) enhances proliferation with 2,4-D and Kinetin (1.5/1.5 mg/l.).

Conclusion: After 42 days of culture initiation and establishment the callus was 520.0±1.12 mg in the mixture of 2,4-D and Kinetin (1.5 mg/l) in Rhus tox free medium. Whereas weight of callus were found to be 1092±0.74 mg after 42 days in the same medium of 2, 4-D and Kinetin (1.5/5.5 mg/l) supplemented with Rhus tox (100 µl/100 ml). Hence, the investigation proponded that the Rhus tox (CH30) has increased the rate of callus development and plantlet regeneration.

Keywords: Rhus toxicodendron (30CH); Scoparia dulcis; micropropagation; leaf explants; IAA; BAP; Kinetin.

1. INTRODUCTION

Scoparia dulcis L. (Family; Scrophulariaceae) is an annual herb commonly called Broomweed or Sweet broom ia a native of tropical America ,but now Pantropical [1]. The experimental data highlights that this plant is an abundant source of phytochemicals such as coumarins, phenols, saponins, tannins, amino acids, flavonoids, catecholamines etc [2]. The HPLC analysis of leaves extract has revealed the presence of two active principles compounds scoparic acid B and scopadulcic acid B [3]. Biologically active compounds like scoparic acid A,C, scopadiol, scopadulcic acid A and scopadulciol and scopadulin has also been isolated from this plant [4] . Hispudulin and β-sitosterol-β-D glucoside has been isolated from the ethanolic extract of whole plant [5] . This enthnomedicinal weed has been indeginously used for the treatment of many health issues like fever, hypertension, stomach ache,headaches, menstrual disorders , jaundice .It has scientifically been proved that the scopadulcic acid B expresses antitumor activity both *in vitro* and *in vivo* [6,7]. Immunoprotective role of *Scoparia dulcis* has been very well elucidated by the aqueous extract of its leaves [8]. Apart from this the experimental plant is endowed with antioxidant and antidiabetic activity [9-11], antiviral [12], analgesic and anti-inflammatory [13] and antibacterial activities [14]. NGF-potentiating activity of *S. dulcis* has been observed due to the presence of acetylated flavones glycosides [15,16]. In addition to these phytochemicals, some other compounds i.e. Adrenaline and Noradrenaline, exhibit sympathomimectic effects [17]. Active ingredients of the herbal plant can be used in drug development either pharmacopoeial, non-pharmacopoeial or synthetic drug. Over exploitation and shrinking natural habitat of useful medicinal plants have brought beneficial herb on the verge of extinction. Moreover large amount of raw materials are required for the herbal drug formulation. But now this problem can be easily solved with the invention of plant tissue culture technique. *In vitro* technique has been sucessfully employed for large scale production of medicinal plants and also in the enhanced production of secondary metabolites in callus [18-21] .Therefore, it is essential to lay down an effective protocol for the clonal propagation and accelerated callus development.In the present study a homeopathic medicine Rhus tox(CH 30) is supplemented into the MS medium. Recently, homeopathic medicines have found its application in the treatment of diseased plants [22-24]. There are also some reports of the use of homeopathic preparations for the enhancement of active principles in medicinal plants [25], increased plant growth rate and metabolism [26]. Preliminary investigation undertaken here might be helpful in legislating a successful protocol for the enhancement of plant growth and callus proliferation.

2. MATERIALS AND METHODS

2.1 Place and Duration of Study

The present work was carried out in Plant Tissue Culture Laboratory, Department of Botany, Patna Science College (Patna University) Patna (India) between September 2017 and August 2018.

2.2 Plant Collection, Identification and Sterilization

The healthy plants of *Scoparia dulcis* (Scrophulariaceae) grown in the herbal garden of Department of Botany, Patna Science College, Patna. Plants were collected and identified following standard monograph (Genera Plantarum of Bentham and Hooker (1962-1883) and used as experimental material. The experiments related to culture was based on the standard methods adopted by Shakti et al. the fresh leaves were excised from the twigs and washed under running tap water for twenty

minutes. The leaves were cut into 2-3 cm pieces. The explants were first washed with 5% savlon solution (v/v) and then with distilled water 4 to 5 times. The surface sterilization was carried out first by immersing in 70% ethanol (v/v) for 45 seconds and then by immersing in 0.1% (w/v) mercuric chloride solution for five minutes and rinsed with sterilized double distilled water in the laminar air flow.

2.3 Explant Sterilization and Incubation

The explants were dried on sterilized filter paper and inoculated in culture tubes (150 X 25 mm) aseptically containing 15-20 ml of solid Murashige and Skoog's medium (MS medium) [27] supplemented with 3% (w/v) sucrose (PCTO607 HIMEDIA), $CaCl_2$, vitamins and 0.8% (w/v) agar (PCT 0901 HIMEDIA), and varied concentrations of auxins viz. Indole 3-acetic acid (IAA), α-naphthalene acetic acid (NAA) and 2,4-dichloro phenoxyacetic acid (2,4-D) and cytokinin viz 6-Benzylaminopurine (BAP) and kinetin in combination with 100µl of Rhus tox 30.pH was adjusted to 5.6-5.8 and the media were sterilized in autoclave at a pressure of 15 lb/square inch and temperature of 121ºC for 15 minutes. The cultures were maintained in the culture room at a temperature of 25±2ºC and relative humidity (RH) of 60-70% at a light intensity of 40-50 $\mu molm^{-2}s^{-1}$ under a photo period of 16/8 hr (light/dark). A minimum of 15 cultures were raised and the experiments were conducted in replicates of three. The cultures were maintained regularly by sub culturing at monthly intervals. The number of explants producing calli was recorded after four weeks of culture. The calli were transferred to fresh media supplemented with BAP, 2, 4-D and Rhus tox for initiation of shoots. The number of shoots produced per callus was recorded in every week. When shoots became 2-3 cm in length they were excised transferred to rooting media vertically in culture tubes containing 15-20 ml of MS medium with different concentrations of Indole 3-acetic acid (IAA) or Indole 3-butyric acid (IBA) or α-napthalene acetic acid (NAA). For each treatment, 25 tubes were inoculated. After 30 days of initial culture, data with respect to cultures producing roots, number of roots per shoots and root length (cm) were recorded. The MS media not supplemented with Rhustox were considered as control. Rooted plantlets were cleaned to remove agar and transferred to sterile earthen pots containing sand and vermiculite (1:1) and covered with polybags with holes. After two weeks plants were acclimatized in culture room without plastic bags for 5-6 hours. One week after acclimatization plants were transferred to pots containing soil under the natural environment for hardening.

2.4 Statistical Analysis

The data for number and length of shoots per explants, and number and length of roots per shoot were statistically analyzed by one way analysis of variance (ANOVA) and significant difference was calculated using Duncan's multiple range tests.

3. RESULTS

3.1 Callus formation

The leaf explants of *Scoparia dulcis* were inoculated in MS medium supplemented with IAA/BAP in the concentration range of 0.1-2.0 mg/l/0.1-1.0 mg/l and the results obtained have been presented in Table-1; Fig-1a-g.

It was observed that the IAA in the concentration range of 0.10 to 2.0 mg/l and BAP in the concentration range of 0.10 to 2.5 mg/l induced callus formation from the leaf explants. IAA in the concentration range of 0.10-0.30 mg/l and BAP in the concentration range of 0.10-0.30 mg/l induced green, compact type of calli. However, IAA in the concentration range of 0.10 to 0.30 and 0.50 mg/l BAP induced the formation of brown friable calli. IAA in the concentration of 0.50 mg/l and 0.10-0.50 mg/l of BAP induced the formation of brown, friable calli. IAA/BAP concentration of 1.50-2.0/1.50-2.5 mg/l induced the formation of green, compact calli. However, equal concentration of IAA and BAP i.e. 2.0 mg/l each induced the formation of brown friable calli (Table-1; Fig: 1a-g).

The effect of 2, 4-D and Kinetin on callus formation was also studied and the results obtained have been presented in Table-2; Fig-2a-g.

The results revealed that when the concentration of 2, 4-D was kept constant i.e. 1.5 mg/l only brown, friable calli were formed at all the concentrations of Kinetin selected in present investigation i.e. from 1.5 mg/l to 6.5 mg/l. However, days of callus induction decreased on increasing the concentration of kinetin. When 2, 4-D and Kinetin concentration were in equal amount i.e. 1.5 mg/l the calli were formed only after 12 days of explants inoculation. The concentration of 2, 4-D/Kinetin in ratio 1.5/2.5

mg/l induced the formation of calli in 10 days; in ratio 1.5/3.5 mg/l in 9 days; in ratio 1.5/4.5-5.5 mg/l in 8 days and in ratio 1.5/6.5 mg/l in 7 days only (Table-2; Fig-2a-g).

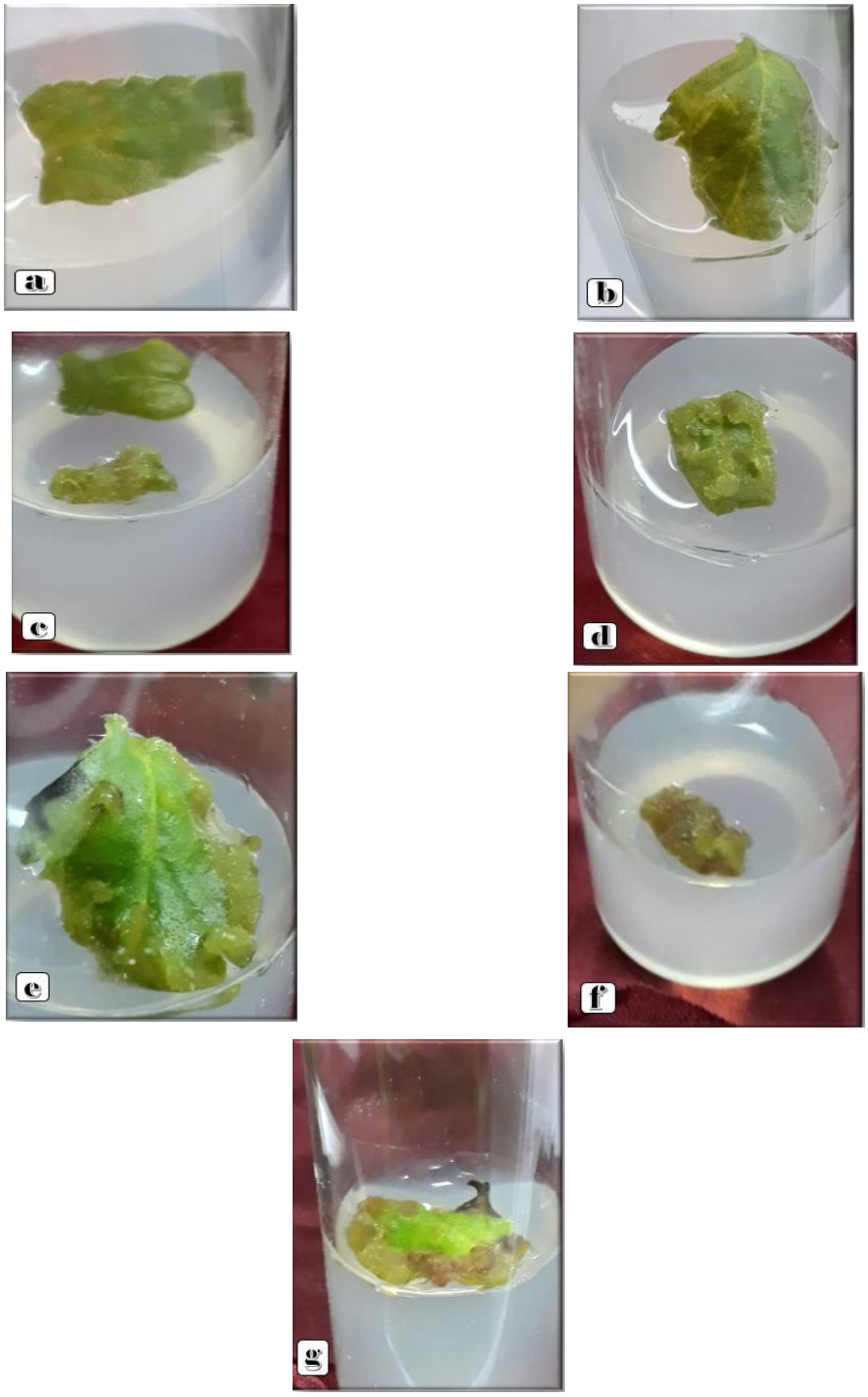

Fig-1a: Induction and growth of callus from leaf explants of *S. dulcis;* b: Induction and growth of callus from leaf explants of *S. dulcis;* c: Development of green, compact callus; d: Development of green, compact callus; e: Development of green, friable callus; f: Development of brown, friable callus; g: Development of brown, friable callus

Table 1. Effect of different concentrations of IAA and BAP in callus induction from leaf explants of *Scoparia dulcis*

Concentration of Phytohormones in mg/l		Callus types
IAA	**BAP**	
0.10	1.0	Green, compact
	0.30	Green, compact
	0.50	Brown, friable
0.20	0.20	Green, compact
0.30	0.10	Green, compact
	0.30	Green, compact
	0.50	Green, friable
0.50	0.10	Brown, friable
	0.30	Brown, friable
	0.50	Brown, friable
1.50	1.50	Green, compact
	2.0	Green, compact
	2.5	Green, compact
2.0	1.50	Green, compact
	2.0	Green, friable
	2.5	Green, compact

Table 2. Effect of 2,4-D and Kinetin on callus formation from leaf explants of *Scoparia dulcis*

Concentration of 2,4-D in mg/l	Concentration of Kinetin in mg/l	Callus types	Days of callus induction
1.5	1.5	Brown, friable	12
1.5	2.5	Brown, friable	10
1.5	3.5	Brown, friable	9
1.5	4.5	Brown, friable	8
1.5	5.5	Brown, friable	8
1.5	6.5	Brown, friable	7

The effect of Rhustox 30 on callus formation from leaf explants on MS media supplemented with various concentrations of 2, 4-D and Kinetin (from 1.5 mg/l to 6.5 mg/l) was studied. MS medium not supplemented with Rhus tox was treated as control.The results obtained have been presented in Tables-3 and 4.

The 2,4-D and Kinetin induced enhanced differentiation of leaf explants cells that caused production of calli in highest amount in comparison to control. A concentration of 2, 4-D/Kinetin in equal amount i.e. 1.5/1.5 mg/l caused production of callus after 10 days of incubation. The weight of calli at this concentration was 12.0±0.65 mg. The weight of calli increased on increasing the days of incubation. After 42 days of incubation the weight of callus was 225.0±1.15 mg. All culture tubes produced the pale yellow and soft calli. The kinetin concentration also influenced enhanced callus production. When 2,4-D/Kinetin concentration was 1.5/2.5 mg/l only nine culture tubes out of ten exhibited calli formation from leaf explants. At this concentration the weight of callus was 601.1±1.13 mg after 42 days of incubation. At concentration of 1.5 mg/l 2,4-D and 3.5-6.5 mg/l Kinetin only eight culture tubes out of ten were responded for callus formation. The calli produced were brown, soft and friable. The maximum weight of callus was obtained at concentration of 2,4-D/Kinetin in ratio 1.5/4.5 mg/l which was 901.3±0.66 mg after 42 days of incubation. Kinetin concentration of more than 4.5 mg/l caused decline in the weight of callus. At concentration of 6.5 mg/l the Kinetin caused reduction in the weight of callus to 880.0±0.63 mg (Table-3).

The homeopathic medicine Rhustox 30 at concentration of 100 µl/100 ml caused enhanced proliferation of leaf explants. All culture tubes responded equally and produced calli only after eight days of inoculation. The calli were brown, soft and friable. At concentration of 2,4-D/Kinetin of 1.5/1.5 mg/l plus Rhustox 30, the weight of callus was 520.0±1.12 mg after 42 days of incubation. The weight of callus increased to

1092±0.74 mg after 42 days of incubation when MS media were inoculated with 2,4-D/Kinetin in concentration of 1.5/5.5 mg/l plus Rhustox 30 (100 µl/100 ml).

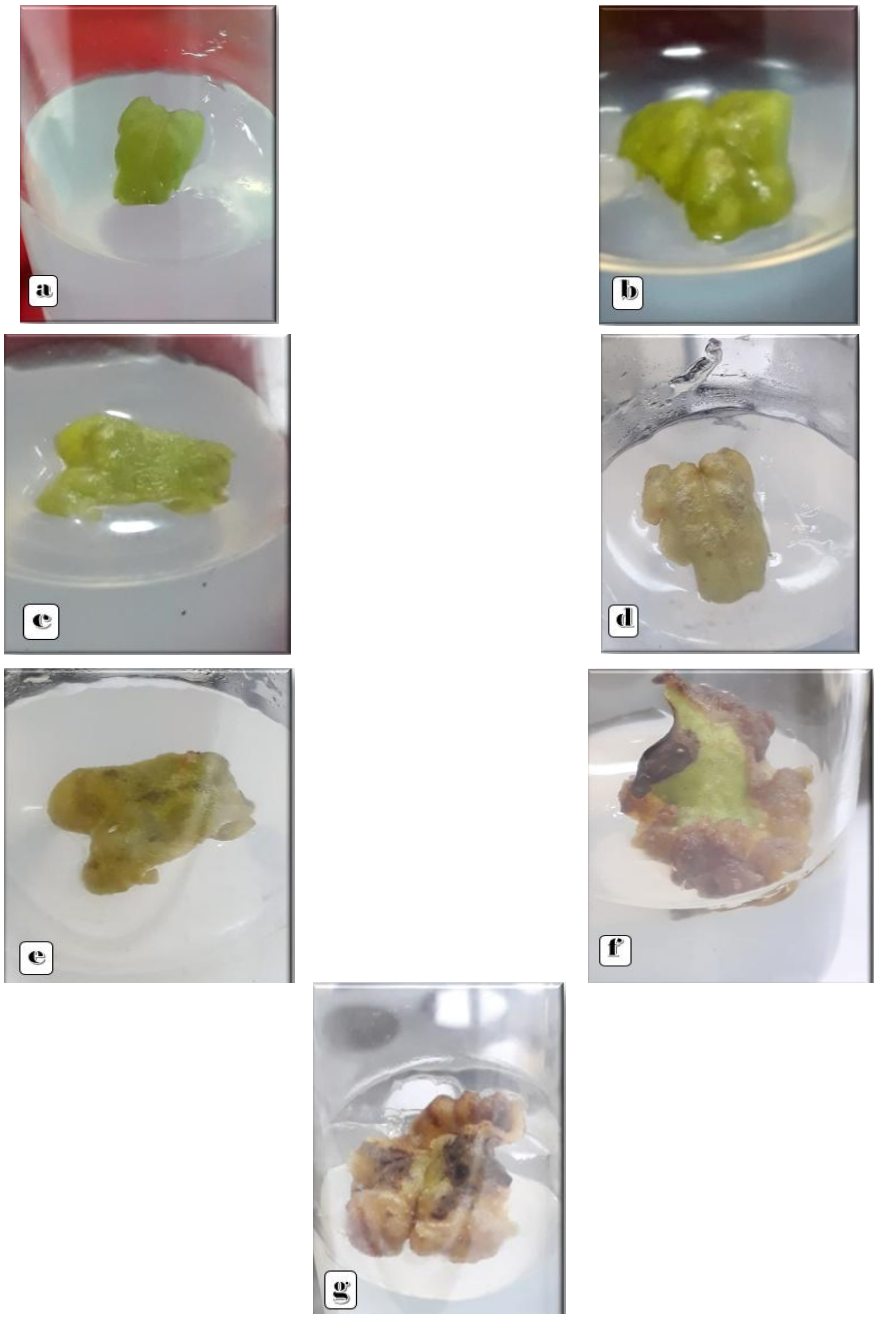

Fig. 2. Different stages of callus development in 2, 4-D/Kinetin supplemented medium; a: Induction of callus from leaf explants; b: Induction of callus from leaf explants after 7 days of inoculation; c: Induction of callus development from leaf explants after 8 days of inoculation d: Formation of brown friable calli after 12 days of inoculation; e: Formation of brown, friable calli after 9 days of inoculation; f: Formation of brown, friable calli after 8 days of inoculation g: Formation of brown, friable calli after 7 days of inoculation

Table 3. Effect of different concentrations of 2,4-D and Kinetin on callus induction from leaf explants of *Scoparia dulcis*

Concentration of 2,4-D/Kinetin in mg/l	Weight of Callus in mg				Response of culture tubes after inoculation out of ten	Minimum number of days for callus induction	Nature of callus
	Days of incubation						
	10	14	28	42			
1.5/1.5	12.0±0.65	50.6±0.45	127.2±0.54	225.0±1.15	All	10	Pale yellow and soft
1.5/2.5	12.9±0.62	52.1±0.75	350.0±0.63	601.1±1.13	9 out of ten	9	Pale yellow to brown soft friable
1.5/3.5	13.1±0.61	51.3±0.47	400.8±0.64	856.8±0.76	All	8	Brown soft friable
1.5/4.5	13.5±0.47	59.2±0.56	425.2±0.81	901.3±0.66	All	8	Brown soft friable
1.5/5.5	13.4±0.35	60.5±0.27	411.4±0.65	889.2±0.71	All	8	Brown soft friable
1.5/6.5	13.5±0.46	55.0±0.48	398.0±0.35	880.0±0.63	All	8	Brown soft friable

Mean ± SE of 25 replicates; results significant at P< 0.05

Table 4. Effect of different concentrations of 2,4-D and Kinetin and Rhustox 30 on callus induction from leaf explants of *Scoparia dulcis*

Concentration of 2,4-D/Kinetin in mg/l	Weight of Callus in mg				Response of culture tubes after inoculation out of ten	Minimum number of days for callus induction	Nature of callus
	Days of incubation						
	10	14	28	42			
1.5/1.5	12.0±0.61	55.4±0.43	150.7±0.44	520.0±1.12	All	8	Brown soft friable
1.5/2.5	12.9±0.64	60.3±0.65	400.1±0.61	658.5±1.16	All	8	Brown soft friable
1.5/3.5	13.1±0.61	55.9±0.43	425.4±0.62	900.3±0.71	All	8	Brown soft friable
1.5/4.5	13.5±0.47	69.5±0.51	403.3±0.71	950.6±0.65	All	8	Brown soft friable
1.5/5.5	13.4±0.35	70.0±0.37	895.4±0.65	1092±0.74	All	8	Brown soft friable
1.5/6.5	13.5±0.46	63.1±0.68	385.0±0.55	700.3±0.61	All	8	Brown soft friable

Mean ± SE of 25 replicates; results significant at P< 0.05

3.2 Shoot Induction

The call i derived from leaf explants were used for shoot induction on MS basal media supplemented with variable concentrations IAA and BAP and the results obtained have been presented in Table-5 and Fig.-3a-e.

The shoot initiation occurred from callus of leaf explants after 7-8 days of transfer to MS media supplemented with variable concentration of IAA and BAP (Table-5). The most effective concentration of IAA and BAP was 1.5 mg each. At this concentration hundred percent shooting was observed in cultures grown after 28 days. The number of shoots and their length were maximum, 3.75±0.12 and 8.65±0.21 respectively with equal amount of IAA and BAP (1.5 mg/l). The percent shoot formation and the length of shoots decreased with increase in concentration of IAA and BAP above 1.5 mg/l. At 2.5 mg/l concentration of IAA and 2.0 mg/l of BAP the shooting was only of 64%. The number of shoots and their length were also minimum, 1.55±0.13 and 4.15±0.13 cm respectively (Table-5; Fig. -3a-e).

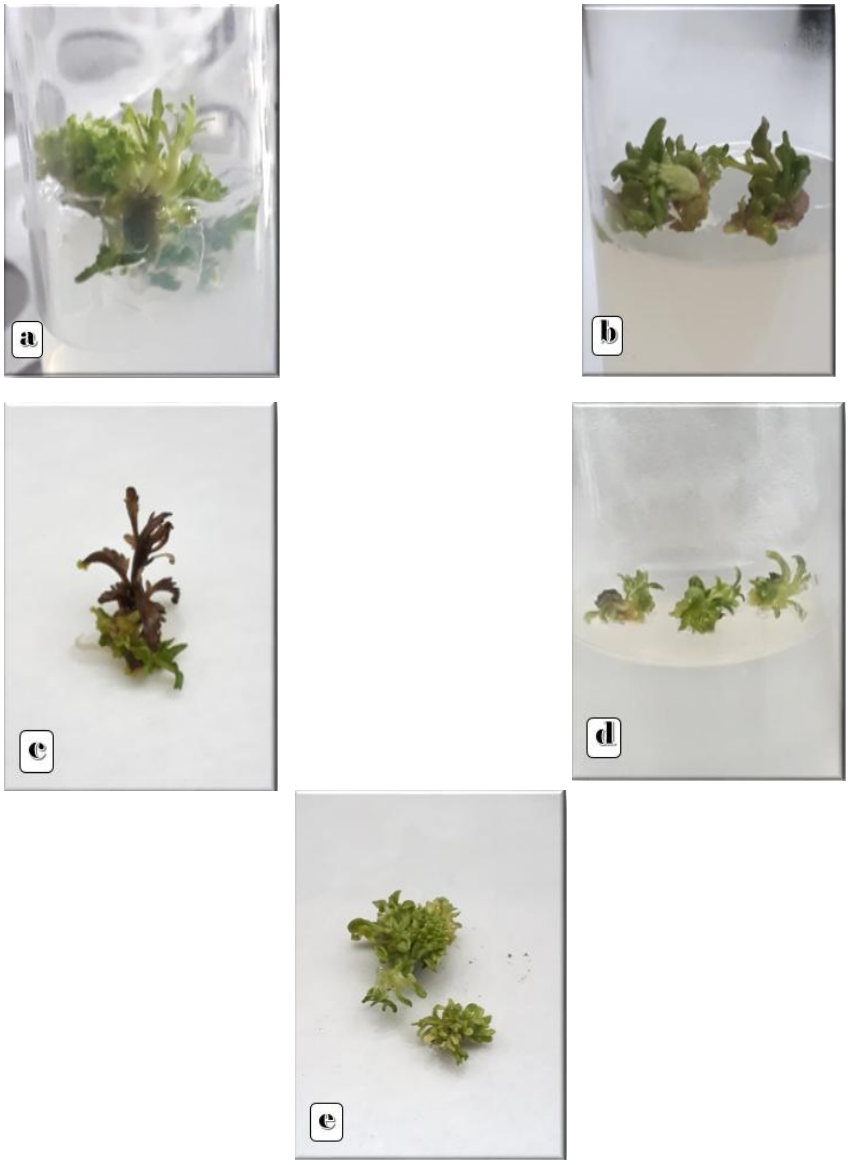

Fig. 3a-e. Different stages of shoot formation in MS media supplemented with different concentrations of IAA and IBA

Table. 5. Effect of different concentrations of IAA and BAP on shoot production from callus of *Scoparia dulcis*

Concentration of IAA in mg/l	Concentration of BAP in mg/l	Number of cultures producing shoots*	Percent shoot formation	Number of Shoots per callus (Ẍ±SE)	Height of Shoots in cm (Ẍ±SE)
1.0	1.0	21	84	2.45±0.15	7.85±0.25
1.5	1.5	25	100	3.75±0.12	8.65±0.21
2.0	1.5	20	80	1.85±0.16	5.16±0.21
2.5	2.0	16	64	1.55±0.13	4.15±0.13
2.5	2.5	17	68	1.57±0.11	4.17±0.12

*Data are Mean ± SE of 25 replicates; * Significant at P= 0.05*

3.3 Root Induction

For root induction shoots of about 3 cm in length were transferred to rooting media containing variable concentrations IBA and IAA separately. For this purpose ½ strength of liquid media and solid MS media were used. Filter Paper Bridge was used in case of liquid media. The results obtained have been presented in Tables-6-9; Fig. 4a-e.

The results revealed that the number of shootlet culture producing roots, percent root formation, number of roots per shootlet and their length increased with increase in concentration of IAA in ½ strength liquid MS media (Table-6). At 1.5 mg/l IAA the root formation started in 7 days of incubation. The number of shooted culture producing roots was 21 out of 25. The percent root formation, number of roots per shoot and their length were 84%, 4.45±0.15 and 3.85±0.35 cm respectively. These values increased on increasing the concentration of IAA up to 4.5 mg/l. At this concentration the rooting started in 7 days of inoculation, and 100% rooting occurred. The number of roots per shoot and their length was 7.45±0.16 and 6.14±0.26 respectively. The percent rooting, number and their length decreased greatly on increasing the concentration of IAA beyond 4.5 mg/l. In solid

Table. 6. Effect of different concentrations of IAA on root production in ½ strength liquid MS media from shoots of *Scoparia dulcis*

Concentration of IAA in mg/l	Days of root induction	Number of cultures producing roots*	Percent root formation	Number of roots per shoot (Ẍ±SE)	Length of roots in cm (Ẍ±SE)
1.5	7	21	84	4.45±0.15	3.85±0.35
2.5	9	22	88	4.75±0.12	4.25±0.37
3.5	8	23	92	5.85±0.11	4.36±0.21
4.5	7	25	100	7.45±0.16	6.14±0.26
5.5	7	18	72	3.57±0.75	3.75±0.34
6.5	6	17	68	2.51±0.21	2.71±0.21

*Data are Mean ± SE of 25 replicates; * Significant at P= 0.05*

Table. 7. Effect of different concentrations of IAA on root production in solid MS media from shoots of *Scoparia dulcis*

Concentration of IAA in mg/l	Days of root induction	Number of cultures producing roots*	Percent root formation	Number of roots per shoot (Ẍ±SE)	Length of roots in cm (Ẍ±SE)
1.5	7	21	84	2.45±0.13	2.87±0.29
2.5	9	21	84	3.75±0.11	3.65±0.21
3.5	8	25	100	5.25±0.16	6.25±0.23
4.5	7	23	92	4.56±0.14	4.25±0.15
5.5	7	20	80	3.57±0.13	3.97±0.16
6.5	6	17	68	2.81±0.17	2.25±0.21

*Data are Mean ± SE of 25 replicates; * Significant at P = 0.05*

MS media a more or less similar results was observed. However, the number of roots produced and their length was comparatively less than the ½ strength liquid media. In solid MS media 100% rooting occurred at 3.5 mg/l of IAA. The number of roots per shoot and their length was 5.25±0.16 and 6.25±0.25 cm respectively (Table-7). A more or less similar result of root induction was noticed in ½ strength liquid MS media and solid MS media supplemented with IBA (Tables-8 and 9).

3.4 Acclimatization of Plantlets

In vitro developed plantlets of about 10-12 cm were subjected to a hardening schedule. The gradual elimination of sucrose and plant growth regulators supported the growth of a well

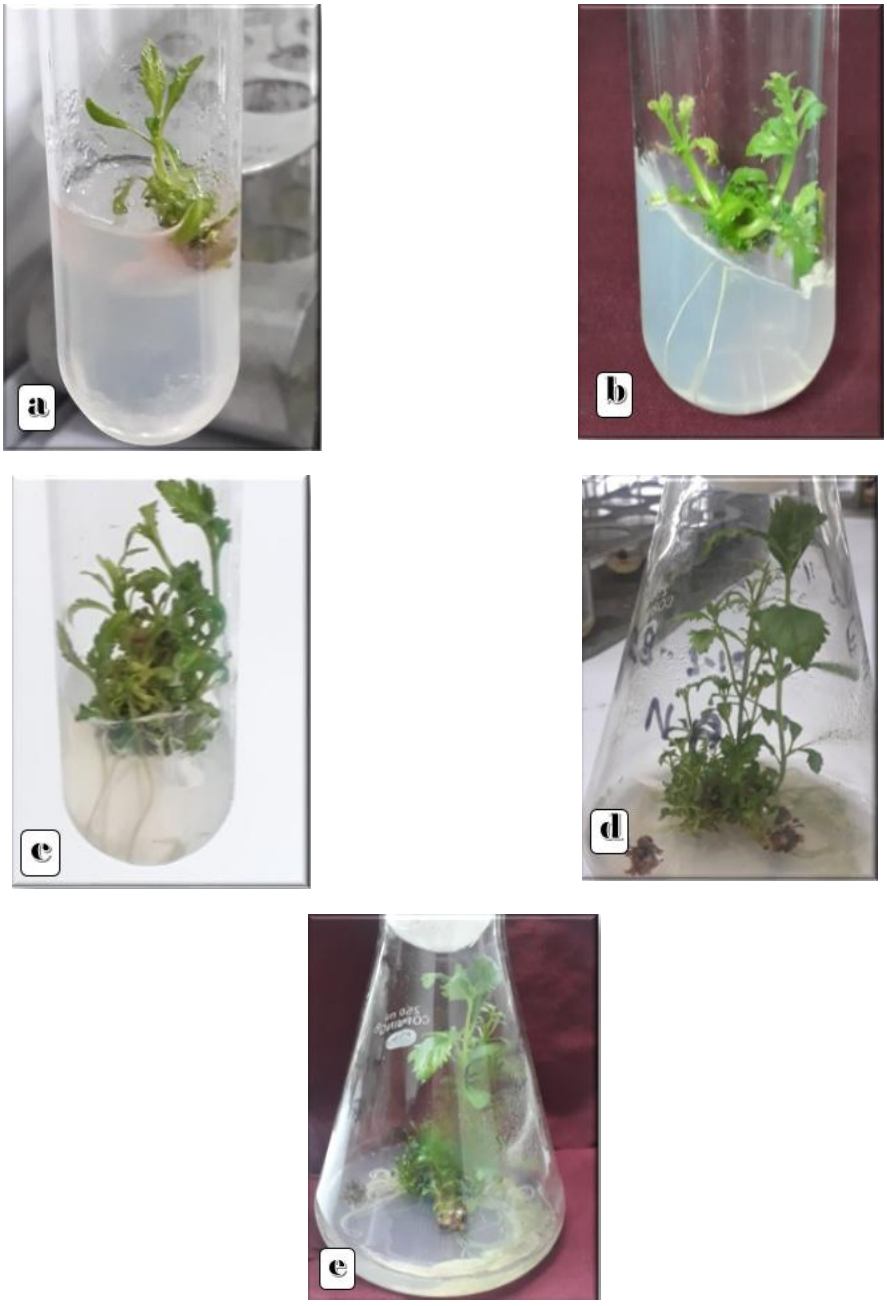

Fig. 4a-e. Different stages of root induction in MS media supplemented with IAA and IBA

Table. 8. Effect of different concentrations of IBA on root production in ½ strength of liquid MS media from shoots of *Scoparia dulcis*

Concentration of IBA in mg/l	Days of root induction	Number of cultures producing roots*	Percent root formation	Number of roots per callus (X̄±SE)	Length of roots in cm (X̄±SE)
1.5	8	21	84	3.65±0.25	3.85±0.26
2.5	8	22	88	3.75±0.22	4.65±0.21
3.5	7	23	92	4.85±0.36	5.16±0.22
4.5	7	25	100	5.65±0.17	6.15±0.23
5.5	7	18	72	3.47±0.18	4.17±0.17
6.5	6	17	68	2.65±0.16	3.75±0.15

*Data are Mean ± SE of 25 replicates; * Significant at P= 0.05*

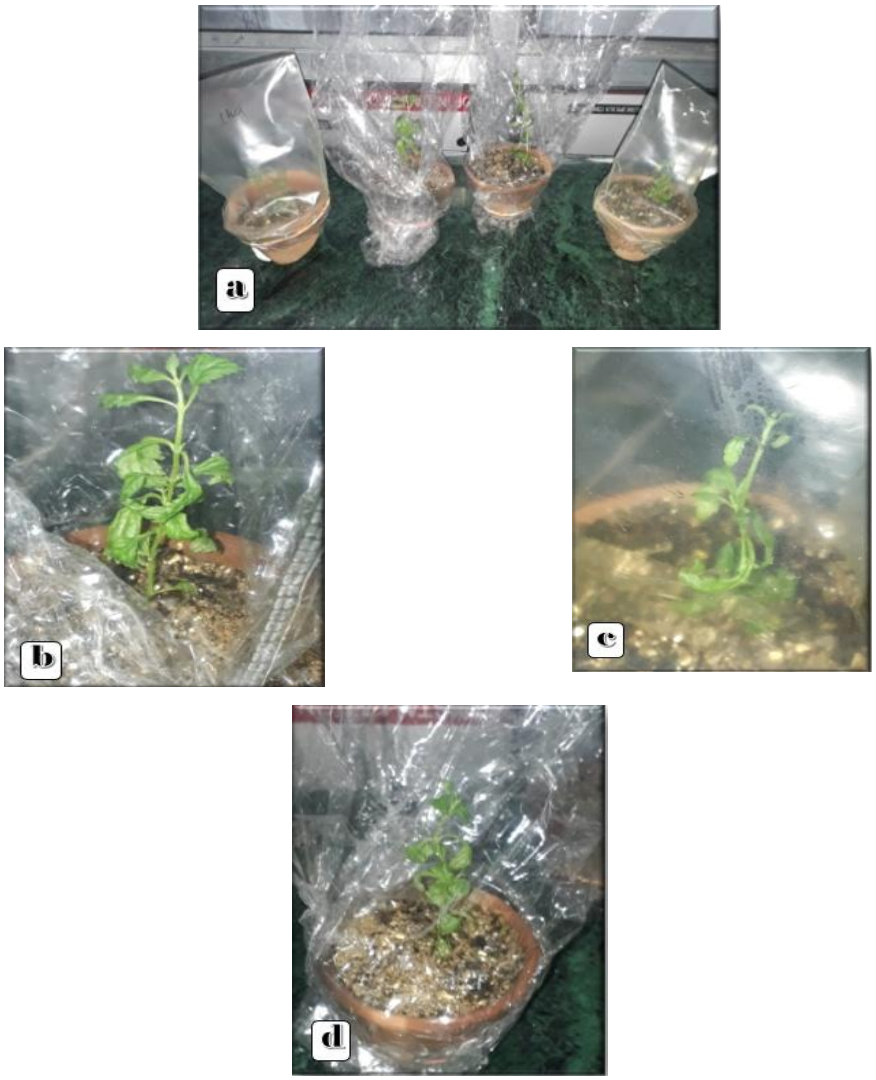

Fig. 5a-d. Different stages of acclimatization processes

balanced shoot and a well balanced root system. The rooted plantlets were transferred to earthen pots containing sand and vermiculite (1:1). The pots were covered with plastic bags and kept in laboratory at 25±2ºC. After 25 days the plants were transferred to soil under natural environment. The survival rate was found to be as much as 88% (Fig-5a-d).

Table. 9. Effect of different concentrations of IBA on root production in solid MS media from shoots of *Scoparia dulcis*

Concentration of IBA in mg/l	Days of root induction	Number of cultures producing roots*	Percent root formation	Number of roots per callus (Ẍ±SE)	Length of roots in cm (Ẍ±SE)
1.5	8	21	84	2.35±0.17	2.75±0.25
2.5	8	21	84	3.65±0.16	2.85±0.25
3.0	7	25	100	5.85±0.12	5.65±0.24
4.5	7	20	80	4.55±0.17	4.25±0.23
5.5	7	17	68	3.57±0.19	4.13±0.16
6.5	6	15	60	2.75±0.21	3.15±0.11

*Data are Mean ± SE of 25 replicates; * Significant at P= 0.05*

4. DISCUSSION

The plant growth regulators viz. auxins (IAA, IBA and NAA) and cytokinins (BAP and Kinetin) were considered more important for micropropagation through leaf explants in some other plants also [28,29].The BAP in combination with IAA or Kinetin in combination with IBA was found suitable for shoot differentiation from callus developed from leaf explants of *Scoparia dulcis*. The present findings gain support from the work of Sakthi and Mohan [30] who studied the micropropagation of *Scoparia dulcis* from their leaf explants and nodal segments and found more or less similar results. A more or less similar result was also noticed by Kothari and Chandra [27-33], Benavides and Caso [34] in case of leaf callus and nodal segments culture of *Tagetes erecta* L.

5. CONCLUSIONS

Micropropagation of *Scoparia dulcis* has been carried out on MS medium supplemented with auxins viz. 2,4-dichloro phenoxy acetic acid (2, 4-D), Indole acetic acid (IAA), Indole butyric acid (IBA) and Cytokinins viz. 6-benzylamino purine (BAP and Kinetin). The elevated callus formation has been seen due to the use of Rhustox 30. The maximum numbers of shoots were produced in the combination of BAP and IAA/ Kinetin and IBA.The transfer of shoots (about 3 cm) to MS solid and ½ strength liquid MS medium favoured rooting in about 28 days and rooted plants (about10 cm) were hardened and established with more than 88% success rate. It can be concluded that the development of micropropagation protocol for medicinally important plant species will facilitate access to the natural and induced variations in near future. In addition to plant growth regulators, Rhustox 30 might be useful in callus formation. It was observed that less number of days were taken for development of callus and it was more in case of Rhustox 30 supplemented media as compared to control. The above observations could be related to the fact that homeopathic medicines has the capability to change the physiological dynamicity by providing the carbon skeleton thereby increasing the biomass. Another relevant fact from the result is that with the increase in callus mass the secondary metabolites content could also be elevated.Thus the bioactive principles responsible for plant growth promotory activity of Rhus tox 30 obtained from *Rhus toxicodendron* L. demands further scrutiny at the scientific level.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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