

3 **Effect of Rhus tox on Micropropagation of *Scoparia dulcis* L.**
4 **through leaf explants culture**

5
6 **ABSTRACT**

7 *Scoparia dulcis* L. is an erect annual herb that shows hypoglycemic, anti tumour promoting, anti viral activities,
8 analgesic and antipyretic activities. The major phytochemicals of this plant include coumarins, phenols, saponins,
9 tannins, amino acids, flavonoids, terpenoids, catecholamines, noradrenaline and adrenaline. The active principles of
10 *Scoparia dulcis* include scoparic acid A, scoparic acid B, scopadulcic acid A and B, scopadulciol and scopadulin.

11 The present study deals with the micropropagation of this plant through leaf explants culture in the MS medium
12 supplemented with different concentration of IAA, IBA, NAA, BAP and Kinetin, and Rhus tox 30, a homeopathic
13 medicine. The results indicated that the IAA in the concentration range of 0.10 to 2.0 mg/l and BAP in the
14 concentration range of 0.10 to 2.5 mg/l induced callus formation from the leaf explants. IAA in the concentration
15 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.
16 However, IAA in the concentration range of 0.10 to 0.30 and 0.50 mg/l BAP induced the formation of brown friable
17 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.
18 IAA/BAP concentration of 1.50-2.0/1.50-2.5 mg/l induced the formation of green, compact calli. 2, 4-D/Kinetin at
19 1.5 mg/l/1.5-6.5mg/l concentrations induced only brown, friable calli. The Rhus tox 30 at concentration of 100µl/l
20 caused enhanced proliferation of leaf explants. At 2,4-D/Kinetin concentration of 1.5/1.5mg/l plus Rhus tox 30, the
21 weight of callus was 520.0±1.12 mg after 42 days of incubation. The weight of callus increased to 1092±0.74 mg
22 after 42 days of incubation with 2,4-D/Kinetin in concentration of 1.5/5.5 mg/l plus Rhus tox 30 (100µl/l). The
23 plantlets were hardened and established with more than 88% success rate.

24 The development of micropropagation protocol will facilitate access to natural and induced variations in near future.
25 In addition to plant growth regulators, Rhus tox 30 might be useful in enhanced callus formation and plantlet
26 regeneration.

27 **Key Words:** *Rhus tox 30, Scoparia dulcis, Micropropagation, Leaf explants, IAA, IBA, α-NAA, BAP, Kinetin*

28
29 **1. INTRODUCTION**

30 *Scoparia dulcis* L. commonly known as Broomweed or Sweet broom belongs to family Scrophulariaceae of
31 Dicotyledonous angiosperm. It is an erect annual herb, measuring about half meter in height and distributed in
32 tropical and subtropical regions. The plant bears serrated leaves and white flowers. Flowers are complete, bisexual,
33 axillary, tetra fid, rotate and measure about 6-7 mm in diameter. Sepals 5, gamosepalous with oval-oblong calyx
34 lobes, three nerved, glabrous, ciliated at the margin and persistent. Petals 5, gamopatelous, pale yellow to white;
35 corolla tube densely hairy at the throat; aestivation imbricate; corona present; corolla lobes 2-4 mm long, apex of
36 petals obtuse, slightly curved, upper lobes slightly larger than others. Stamens 4 exerted; filament inserted at the top
37 of the corolla tube; anthers dorsifixed. Carpels 2, syncarpous, ovary superior, unilocular; ovules anatropous,

38 numerous on axile placentation; style erect, about 2 mm long; stigma truncate to bipartite. Flowering time is
39 throughout the year.

40 The ethnomedicinal uses of *Scoparia dulcis* are well documented [1] and show hypoglycemic, anti tumour
41 promoting, anti viral activities, analgesic and antipyretic activities [2-8]. *Scoparia dulcis* is rich in flavonoids and
42 terpenes and pharmacological properties of this plant is due to the presence of these phytochemicals [9].

43 The major phytochemicals of this plant include coumarins, phenols, saponins, tannins, amino acids, flavonoids,
44 terpenoids and catecholamines [10]. Noradrenaline and adrenaline are also present in addition to these
45 phytochemicals [11] which possess sympathomimetic effects. The active principles of *Scoparia dulcis* include
46 scoparic acid A, scoparic acid B, scopadulcic acid A and B, scopadulciol and scopadulin [12, 13]. These active
47 principles possess inhibitory activity against replication of herpes simplex virus (HSV), gastric H⁺, K⁺ ATPase
48 activator and antitumor promoting activity [12]. The terpenoids viz. alpha-amyrin, betulinic acid, dulcioic acid,
49 friedelin, glutinol and ifflaionic acid have also been identified [14-16].

50
51 Pharmacological activities of *Scoparia dulcis* have been investigated and known to possess antidiabetic activity
52 [17, 18, 19] (Pari et al., 2004; Latha and Pari, 2004; 2005), analgesic and antitumour activity, antiviral activity [20-
53 22] (Hayashi *et al.*, 1988, 1990, 1992), antimalarial activity [23] (Riel et al., 2002), neurotrophic activity [24, 25] (Li
54 and Ohizum, 2004; Li et al., 2004), anticancer activity [26, 27] (Nkembo et al., 2005; Nakagiri et al., 2005), diuretic
55 activity [28, 19] (Ahmed et al., 2001; Freire et al., 1991).

56 Plant tissue culture is being used for the improvement and rapid multiplication of medicinal plants [29, 30].
57 *Scoparia dulcis* is recognized as medicinal plant and the present study deals with the micropropagation of this plant
58 through leaf explants culture in the MS medium supplemented with 100 µ/ml of Rhus tox 30, a homeopathic
59 medicine obtained from a plant *Rhus toxicodendron* Linn. (Poison ivy) of family Anacardiaceae. Rhus tox is used in
60 the treatment of cramps, strains, sprains, restless leg syndrome, flu, viral infections and arthritis.

61 2. MATERIALS AND METHODS

62 2.1. Place and Duration of Study

63 The present work was carried out in Plant Tissue Culture Laboratory, Department of Botany, Patna Science College
64 (Patna University) Patna (India) between September 2018 and August 2019.

65 2.2 Plant Collection, Identification and Sterilization

66 The healthy plants of *Scoparia dulcis* (Scrophulariaceae) grown in the herbal garden of Department of Botany, Patna
67 Science College, Patna were collected and identified following standard monographs and used as experimental
68 material. The fresh leaves were excised from the twigs and washed under running tap water for twenty minutes. The
69 leaves were cut into 2-3 cm pieces. The explants were first washed with 5% savlon solution (v/v) and then with
70 distilled water 4 to 5 times. The surface sterilization was carried out first by immersing in 70% ethanol (v/v) for 45
71 seconds and then by immersing in 0.1% (w/v) mercuric chloride solution for five minutes and rinsed with sterilized
72 double distilled water in the laminar air flow.

73

74 2.3 Explant Sterilization and Incubation

75 The explants were dried on sterilized filter paper and inoculated in culture tubes (150 X 25 mm) aseptically
76 containing 15-20 ml of solid Murashige and Skoog's medium (MS medium) [31] supplemented with 3% (w/v)
77 sucrose (PCTO607 HIMEDIA), CaCl₂, vitamins and 0.8% (w/v) agar (PCT 0901 HIMEDIA), and varied
78 concentrations of auxins viz. Indole 3-acetic acid (IAA), α-naphthalene acetic acid (NAA) and 2,4-dichloro phenoxy

79 acetic acid (2,4-D) and cytokinin viz 6-Benzylamino purine (BAP) and kinetin in combination with 100µl of Rhus
80 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
81 temperature of 121⁰C for 15 minutes. The cultures were maintained in the culture room at a temperature of 25±2⁰C
82 and relative humidity (RH) of 60-70% at a light intensity of 40-50 µmolm⁻²s⁻¹ under a photo period of 16/8 hr
83 (light/dark). A minimum of 15 cultures were raised and the experiments were conducted in replicates of three. The
84 cultures were maintained regularly by sub culturing at monthly intervals. The number of explants producing calli
85 was recorded after four weeks of culture. The calli were transferred to fresh media supplemented with BAP, 2, 4-D
86 and Rhus tox for initiation of shoots. The number of shoots produced per callus was recorded in every week. When
87 shoots became 2-3 cm in length they were excised transferred to rooting media vertically in culture tubes containing
88 15-20 ml of MS medium with different concentrations of Indole 3-acetic acid (IAA) or Indole 3-butyric acid (IBA)
89 or α-napthalene acetic acid (NAA). For each treatment, 25 tubes were inoculated. After 30 days of initial culture,
90 data with respect to cultures producing roots, number of roots per shoots and root length (cm) were recorded. The
91 MS media not supplemented with Rhus tox were considered as control. Rooted plantlets were cleaned to remove
92 agar and transferred to sterile earthen pots containing sand and vermiculite (1:1) and covered with polybags with
93 holes. After two weeks plants were acclimatized in culture room without plastic bags for 5-6 hours. One week after
94 acclimatization plants were transferred to pots containing soil under natural environment for hardening.

95 2.4 Statistical Analysis

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

99 3. RESULTS

100 3.1. Callus formation

101 The leaf explants of *Scoparia dulcis* were inoculated in MS medium supplemented with IAA/BAP in the
102 concentration range of 0.1-2.0 mg/l/0.1-1.0 mg/l (Table-1).

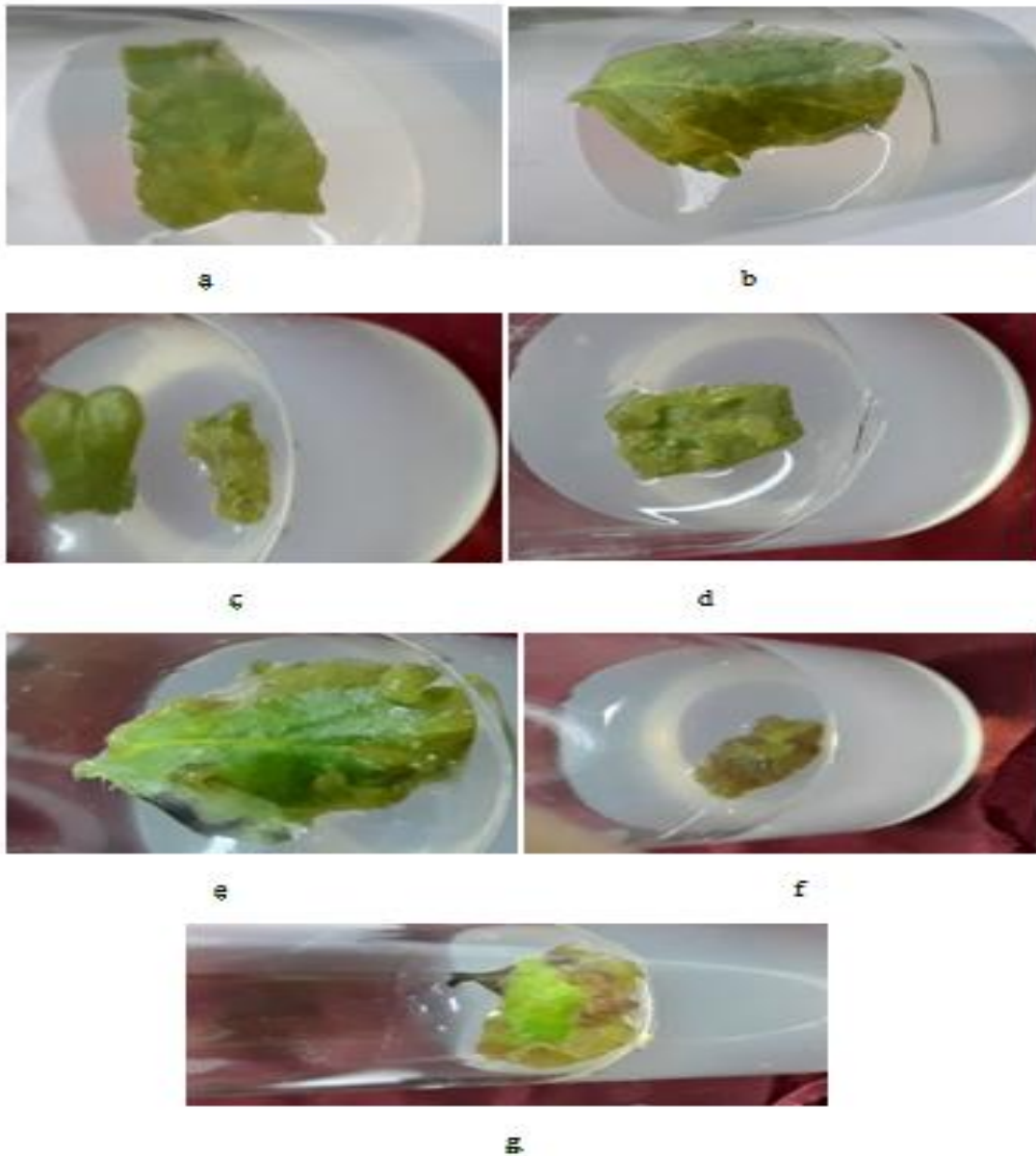
103 **Table-1: Effect of different concentrations of IAA and BAP in callus induction from leaf explants of**
104 ***Scoparia dulcis***

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

105

106 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
107 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
108 and BAP in the concentration range of 0.10-0.30 mg/l induced green, compact type of calli. However, IAA in the
109 concentration range of 0.10 to 0.30 and 0.50 mg/l BAP induced the formation of brown friable calli. IAA in the
110 concentration of 0.50 mg/l and 0.10-0.50 mg/l of BAP induced the formation of brown, friable calli. IAA/BAP
111 concentration of 1.50-2.0/1.50-2.5 mg/l induced the formation of green, compact calli. However, equal
112 concentration of IAA and BAP i.e. 2.0 mg/l each induced the formation of brown friable calli (Table-1; Fig: 1a-g).

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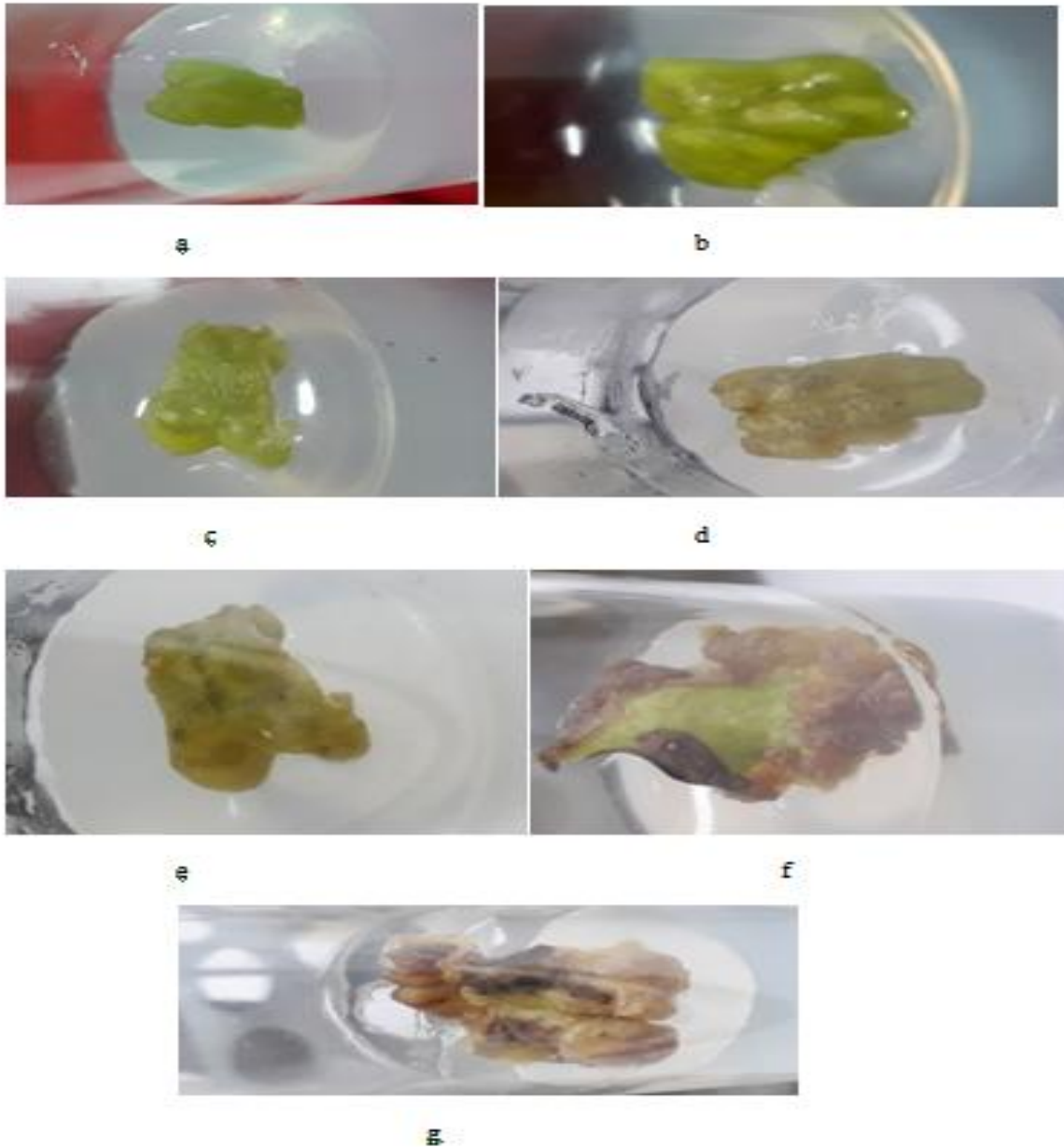
- 119
 120 Fig-1a: Induction and growth of callus from leaf explants of *S. dulcis*
 121 Fig-1b: Induction and growth of callus from leaf explants of *S. dulcis*
 122 Fig-1c: Development of green, compact callus
 123 Fig-1d: Development of green, compact callus
 124 Fig-1e: Development of green, friable callus
 125 Fig-1f: Development of brown, friable callus
 126 Fig-1g: Development of brown, friable callus
 127

128 The effect of 2, 4-D and Kinetin on callus formation was also studied and the results obtained have been presented
 129 in Table-2

130 **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

131
 132 From the result it was observed that when the concentration of 2, 4-D was kept constant i.e. 1.5 mg/l only brown,
 133 friable calli were formed at all the concentrations of Kinetin selected in present investigation i.e. from 1.5 mg/l to
 134 6.5 mg/l. However, days of callus induction decreased on increasing the concentration of kinetin. When 2, 4-D and
 135 Kinetin concentration were in equal amount i.e. 1.5 mg/l the calli were formed only after 12 days of explants
 136 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
 137 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-
 138 2a-g).



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141 Fig-2: Different stages of callus development in 2, 4-D/Kinetin supplemented medium

142 Fig-2a: Induction of callus from leaf explants

143 Fig-2b: Induction of callus from leaf explants after 7 days of inoculation

144 Fig-2c: Induction of callus development from leaf explants after 8 days of inoculation

145 Fig-2d: Formation of brown friable calli after 12 days of inoculation

146 Fig-2e: Formation of brown, friable calli after 9 days of inoculation

147 Fig-2f: Formation of brown, friable calli after 8 days of inoculation

148 Fig-2g: Formation of brown, friable calli after 7 days of inoculation

149

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

153 **Table-3: Effect of different concentration of Kinetin on callus induction from leaf explants of**
 154 ***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

155

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

157 **Table-4: Effect of different concentration of Kinetin and Rhus tox 30 on callus induction from leaf**
 158 **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

159

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

161

162 From the results it is evident that the Kinetin induced enhanced dedifferentiation of leaf explants cells that caused
163 production of calli in highest amount in comparison to control. A concentration of 2, 4-D/Kinetin in equal amount
164 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
165 12.0 ± 0.65 mg. The weight of calli increased on increasing the days of incubation. After 42 days of incubation the
166 weight of callus was 225.0 ± 1.15 mg. All culture tubes produced the pale yellow and soft calli. The kinetin
167 concentration also influenced enhanced callus production. When 2,4-D/Kinetin concentration was 1.5/2.5mg/l only
168 nine culture tubes out of ten exhibited calli formation from leaf explants. At this concentration the weight of callus
169 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
170 eight culture tubes out of ten were responded for callus formation. The calli produced were brown, soft and friable.
171 The maximum weight of callus was obtained at concentration of 2,4-D/Kinetin in ratio 1.5/4.5 mg/l which was
172 901.3 ± 0.66 mg after 42 days of incubation. Kinetin concentration of more than 4.5 mg/l caused decline in the weight
173 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-
174 3).

175 The homeopathic medicine Rhus tox 30 at concentration of 100µl/l caused enhanced proliferation of leaf explants.
176 All culture tubes responded equally and produced calli only after eight days of inoculation. The calli were brown,
177 soft and friable. At concentration of 2,4-D/Kinetin of 1.5/1.5mg/l plus Rhus tox 30, the weight of callus was
178 520.0 ± 1.12 mg after 42 days of incubation. The weight of callus increased to 1092 ± 0.74 mg after 42 days of
179 incubation when MS media were inoculated with 2,4-D/Kinetin in concentration of 1.5/5.5 mg/l plus Rhus tox 30
180 (100µl/l).

181 3.2. Shoot induction

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

184 **Table-5: Effect of different concentrations of IAA and BAP on shoot production from callus of**
185 ***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 ($\bar{X} \pm SE$)	Height of Shoots in cm ($\bar{X} \pm SE$)
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

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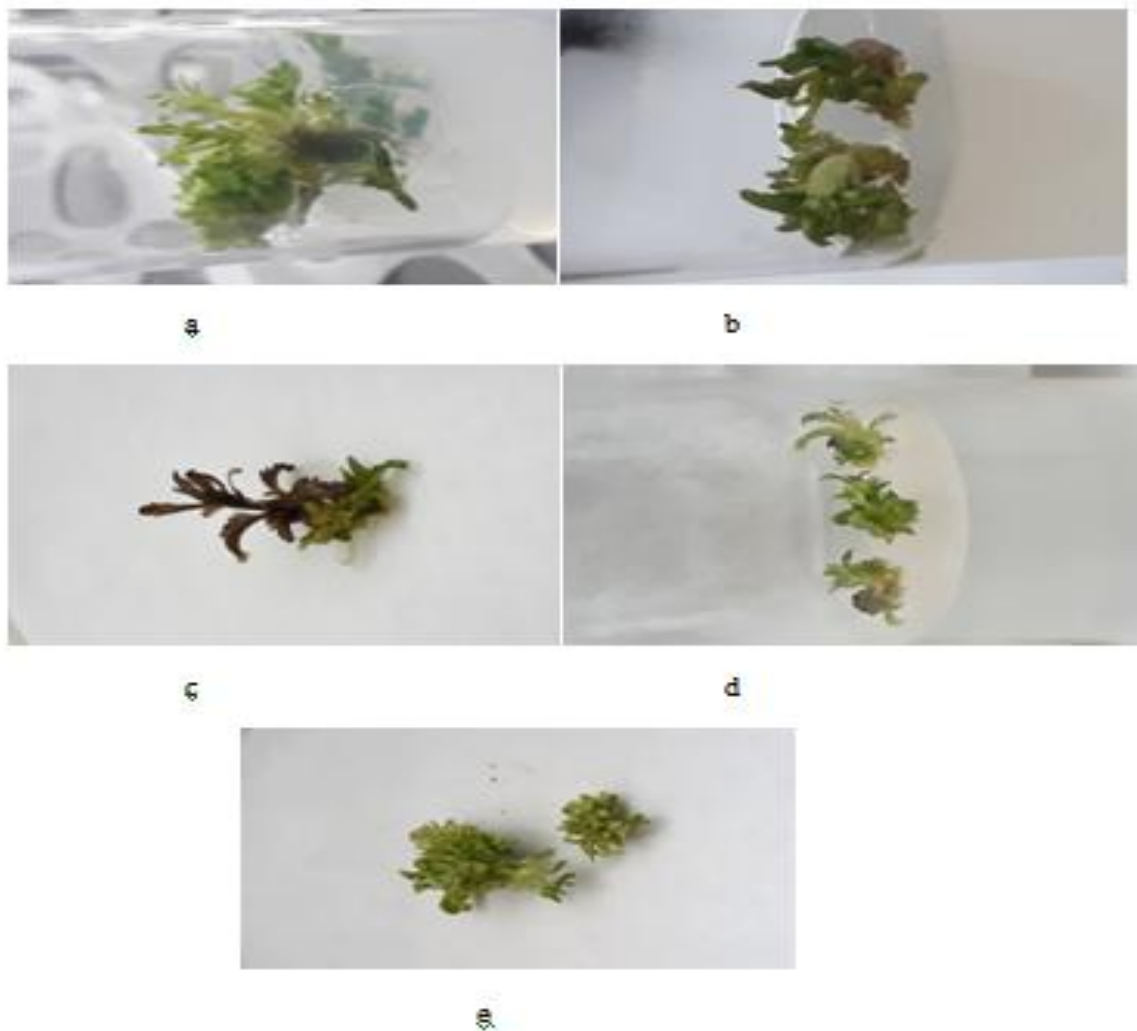
187 Data are Mean \pm SE of 25 replicates; * Significant at $P = 0.05$

188 From the results it is evident that the shoot initiation occurred from callus of leaf explants after 7-8 days of transfer
189 to MS media supplemented with variable concentration of IAA and BAP (Table-5). The most effective
190 concentration of IAA and BAP was 1.5 mg each. At this concentration hundred percent shooting was observed in
191 cultures grown after 28 days. The number of shoots and their length were maximum, 3.75 ± 0.12 and 8.65 ± 0.21
192 respectively with equal amount of IAA and BAP (1.5 mg/l). The percent shoot formation and the length of shoots
193 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

194 mg/l of BAP the shooting was only of 64 %. The number of shoots and their length were also minimum, 1.55 ± 0.13
195 and 4.15 ± 0.13 cm respectively (Table-5; Fig-3a-e).

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

202 **3.3. Root induction**

203 For root induction shoots of about 3 cm in length were transferred to rooting media containing variable
204 concentrations IBA and IAA separately. For this purpose $\frac{1}{2}$ strength of liquid media and solid MS media were used.
205 Filter Paper Bridge was used in case of liquid media. The results obtained have been presented in Table-6-9; Fig 4a-
206 e.

207 **Table-6: Effect of different concentrations of IAA on root production in ½ strength liquid MS**
 208 **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 ($\bar{X} \pm SE$)	Length of roots in cm ($\bar{X} \pm SE$)
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

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

211 **Table-7: Effect of different concentrations of IAA on root production in solid MS media from**
 212 **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 ($\bar{X} \pm SE$)	Length of roots in cm ($\bar{X} \pm SE$)
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

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

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225 **Table-8: Effect of different concentrations of IBA on root production in ½ strength of liquid MS**
 226 **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 ($\bar{X} \pm SE$)	Length of roots in cm ($\bar{X} \pm SE$)
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

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

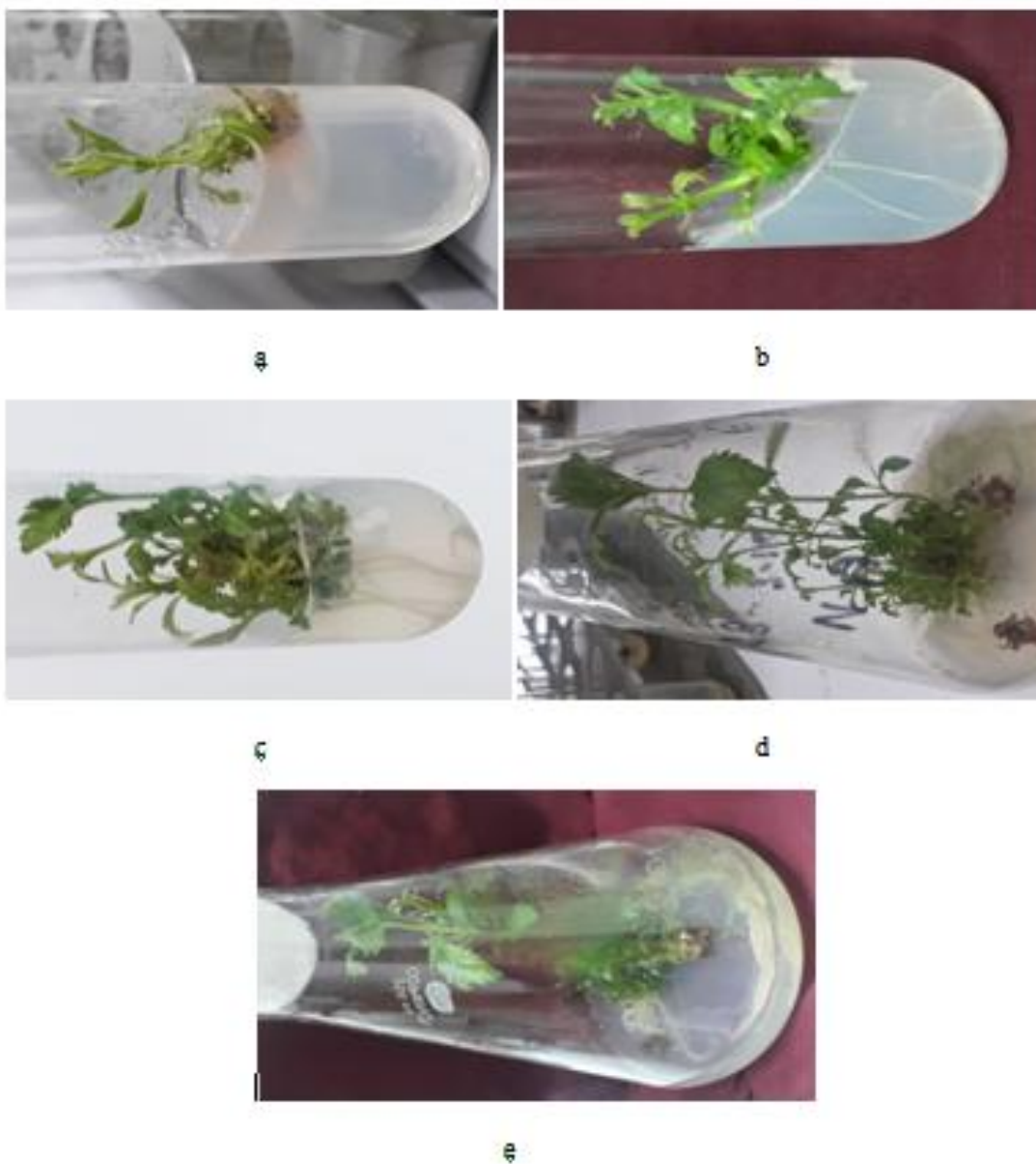
229 **Table-9: Effect of different concentrations of IBA on root production in solid MS media from**
 230 **shoots of *Scoparia dulcis***

Concentration of IBA in mg/l	Days of root induction	Number of cultures producing roots*	Percent root formation	Number of roots per callus ($\bar{X} \pm SE$)	Length of roots in cm ($\bar{X} \pm SE$)
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

231

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

233 From the results (Table-6) it is evident that the number of shoot let culture producing roots, percent root formation,
 234 number of roots per shoot let and their length increased with increase in concentration of IAA in ½ strength liquid
 235 MS media. At 1.5 mg/l IAA the root formation started in 7 days of incubation. The number of shootlet culture
 236 producing roots was 21 out of 25. The percent root formation, number of roots per shoot and their length were 84%,
 237 4.45±0.15 and 3.85±0.35 cm respectively. These values increased on increasing the concentration of IAA up to 4.5
 238 mg/l. At this concentration the rooting started in 7 days of inoculation. All the shoots produced roots i.e. 100%
 239 rooting occurred. The number of roots per shoot and their length was 7.45±0.16 and 6.14±0.26 respectively. The
 240 percent rooting, number and their length decreased greatly on increasing the concentration of IAA beyond 4.5 mg/l.
 241 In solid MS media a more or less similar results was observed. However, the number of roots produced and their
 242 length was comparatively less than the ½ strength liquid media. In solid MS media 100% rooting occurred at 3.5
 243 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-
 244 7). A more or less similar result of root induction was noticed in ½ strength liquid MS media and solid MS media
 245 supplemented with IBA (Table-8 and 9).



246

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

248 3.4. Acclimatization of Plantlets

249 *In vitro* developed plantlets of about 10-12 cms were subjected to a hardening schedule. The gradual elimination of
 250 sucrose and plant growth regulators supported the growth of a well balanced shoot and a well balanced root system.
 251 The rooted plantlets were transferred to earthen pots containing sand and vermiculite (1:1). The pots were covered
 252 with plastic bags and kept in laboratory at $25\pm 2^{\circ}\text{C}$. After 25 days the plants were transferred to soil under natural
 253 environment. The survival rate was found to be as much as 88% (Fig-5a-d).

254

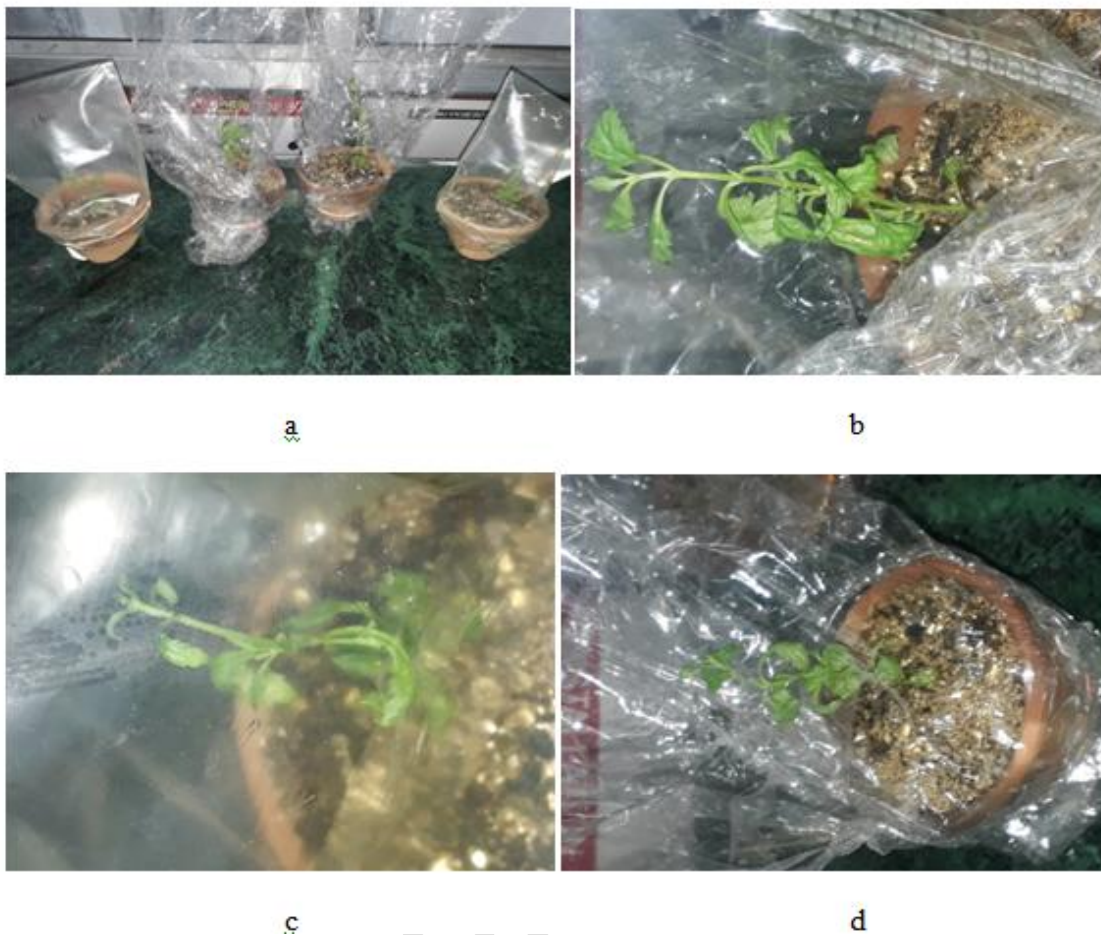


Fig-5a-d: Different stages of acclimatization processes

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 [32, 33]. 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 [34] 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 [35-38], Benavides and Caso [39], Gita Rani *et al.*, [40] in case of leaf callus and nodal segments culture of *Tagetes erecta* L.

5. CONCLUSIONS

Micropropagation of *Scoparia dulcis* was 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 homeopathic medicine Rhus tox induced enhanced formation of callus from leaf explants. The maximum numbers of shoots were produced in the combination of BAP and IAA/ Kinetin and IBA. Transfer of shoots (about 3 cm) to MS solid and and ½ strength liquid MS medium favoured rooting in about 28 days and rooted plants (about 10cm) 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

274 access to natural and induced variations in near future. In addition to plant growth regulators, Rhus tox 30 might be
275 useful in callus formation. The bioactive principles responsible for plant growth promotory activity of Rhus tox 30
276 obtained from *Rhus toxicodendron* L. demands further investigation at scientific level.
277

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