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Micropropagation of medicinal herb, *Hypochaeris radicata* L. via indirect organogenesis

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***Hypochaeris radicata* is a perennial herb, commonly called as 'hairy cat's-ear'. The edible leaves and juice form of roots are suggested by the local healers of Nilgiris, Tamil Nadu, India for the treatment of inflammation and infectious diseases and for other health benefits. They were exploiting this plant severely, for the preparation of herbal formulations. The present study was aimed at to establish the *H. radicata* through *in vitro* cultures using *in vitro* derived leaf explants for conservation of this species in natural habitats. Effective callus formation was noted in the MS medium fortified with BAP and NAA at 3.0 and 1.0 mg/L respectively (98.83%) followed by BAP and NAA at 3.0 and 0.5mg/L respectively (93.33%) and 2,4-D at 0.2mg/L (96.36%). The colour of the callus was yellowish green produced in MS medium with BAP and NAA and pale yellow in 2,4-D. The *in vitro* derived calli were well responded for shoot formation (94.25%) and root (87.65%) formations in BAP and NAA at 3.0 and 1.0mg/L respectively. The *in vitro* regenerated plantlets were successfully acclimatized (72.54%) in the hardening medium containing garden soil: sand: vermicompost of 1:1:1 by volume. The described protocol could be useful for germplasm conservation and commercial cultivation of *H. radicata*.**

Keywords: *Hypochaeris radicata,* Asteraceae, *in vitro* regeneration, *in vitro* derived leaf explants.

Abbreviations: BAP-6-Benzyladenine, NAA-Napthalene acetic acid, IAA-Indole acetic acid, 2,4-D-2,4-Dichlorophenoxy acetic acid, MS-Murashige and Skoog.

Hypochaeris radicata, an edible, perennial herb, belongs to the family Asteraceae is commonly called as 'hairy cat's-ear'. It is native to South Africa and distributed in forest margins of Nilgirs, the Western Ghats, Tamil Nadu at 2000m above msl. It possess several medicinal properties such as anti-inflammatory, anticancer, antioxidant, antibacterial, antifungal (Jamuna *et al.,* 2012, 2013) and antidiuretic. It is being used for the treatment of jaundice, rheumatism, dyspepsia, constipation, hypoglycemia and kidney related problems in traditional medicinal practice of Tamil Nadu, India (Pullaiah, 2006). It is used for medicinal purpose in Meghalaya (Tynsong *et al.,* 2004) and used as food for ruminants in British Columbia (Cherly *et al.,* 2007). It was reported to have many bioactive compounds (Jamuna and Paulsamy, 2013). The leaves are usually blanched, steamed and cooked like any other leafy vegetable. Salads are made with the leaves, raw as well as boiled or steamed. The flowers can be battered and made into fritters. A number of dishes are flavored with the petals of the flowers, which are also added to sautéed dishes. In Greece and Crete, leaves of this plant are eaten raw, along with other similar herbs such as pachies and agrioradika. Alcoholic beverages such as wine are flavored with

the petals of these flowers. The roots of this herb are ground and roasted and used as a substitute for coffee (www.ifood.tv/network/catsear).

Due to the over exploitation for its medicinal uses, the species become lower in population size in high hills of Nilgiris (Padmavathy *et al.,* 2008). Propagation of this species is hampered by low seed viability and hence the lower germination rate (<3%) also (Josep and Evelyn, 2007; Paulsamy *et al.,* 2008). To address this lacuna an attempt was made to develop an efficient microprogation protocol by using *in vitro* derived leaf explants.

Materials and Methods

Collection of the plant materials

The seeds of the study species, *Hypochaeris radicata* were collected during the month of February, 2013 at Kattabettu, Nilgiris, the Western Ghats, Tamil Nadu, India. The authenticity of the plant was confirmed in Botanical Survey of India, Southern Regional Circle, Coimbatore by referring the deposited specimen. The voucher number of the specimen is BSI/SRC/5/23/2010-11/Tech.153.

Sterilization of seeds

The seeds were washed with running tap water for 15minutes followed by 3-4 rinses in double distilled water. These seeds were sterilized with 0.1% (w/v) $HgCl_2$ for 5 minutes followed by 4-5 rinses with sterilized double distilled water.

Preparation of MS medium and culture conditions

The MS medium (Murashige and Skoog, 1962) containing 30% sucrose solidified with 8% agar (Tissue culture grade, Hi-Media, India) and the pH of the medium was adjusted to 5.6-5.8 with 0.1 N NaOH or 0.1 N HCl prior to the addition of agar. The medium was autoclaved at 121°C and 15lbf/Inch^2 pressure for 15 minutes. The cultures were maintained under white fluorescent light having 2000 lux light intensity. The incubation temperature was 25±2°C with 16 hours light and 8 hours dark period in every 24 hours cycle.

Seed germination

The surface sterilized seeds were inoculated in the test tubes containing MS medium without any addition of growth regulators. After the germination of seeds the cotyledonary leaf portion was removed and cultured on a MS medium containing various growth hormones.

Callus induction

The *in vitro* derived cotyledonary leaf explants were inoculated on MS medium supplemented with different concentrations and combinations of growth regulators *viz.*, BAP, NAA, 2,4-D and IAA. The days required for callus formation, per cent response of explants, colour and texture of the callus was observed.

Shoot regeneration

For the shoot induction, the *in vitro* derived callus was transferred to MS medium containing different concentrations and combinations of growth regulators such as BAP, NAA and IAA. The percentage of explants responding, number of shoots per callus and length of the shoots were recorded after 40 days of culture.

Root induction and acclimatization

The proliferated shoots were transferred to MS medium fortified with various concentrations of BAP, NAA and IAA for root formation. The rooting attributes *viz.*, per cent shoots responding for rooting, number of roots per shoot and root length was observed. The rooted plantlets were transferred to the hardening medium containing various combinations of hardening mixtures and the rate of survivability was determined.

Statistical analysis

For this *in vitro* regeneration technique, thirty explants were used for each culture and the experiments were repeated thrice. These data were subjected to statistical analysis (ANOVA) and means

of different experiments were compared by using Duncan's Multiple Range Test (p<0.05) (Duncan, 1955).

Results

Callus induction

The percentage of callus formation was noted to be varied widely across the MS medium containing different concentrations and combinations of the growth regulators, BAP, NAA, 2,4-D and IAA (Table 1). The highest percentage of 98.83% explants derived callus in MS medium supplemented with BAP and NAA at 3.0 and 1.0mg/L respectively (yellowish green and friable) followed by 2,4-D at 0.2mg/L (96.36% pale yellow and friable) and BAP and NAA at 3.0 and 0.5mg/L respectively (93.33% yellowish green and friable) [Fig. 1(a)]. The colour and nature of the callus was widely varied with different concentrations and combinations of growth regulators tried. Green colour of the callus was observed on MS medium fortified with lower concentrations of BAP (0.5, 1.0, 1.5 and 2.0mg/L) and increasing of concentrations of BAP at ≤3.0mg/L has changed the callus into brown colour and hard. Yellowish green colour callus formation with friable nature was noted in all the concentrations of BAP and NAA. It was noted that the colour and texure of the callus were changed according to the increasing of concentrations of 2,4-D with concomitant decrease of percent response of explants for callus formation from 96.36% (0.2mg/L) to 19.04% (3mg/L) [Fig. 1(e,f)].

Shoot regeneration

The *in vitro* derived callus was subcultured onto the MS medium consisting of the growth regulators, BAP, NAA and IAA for further organogenesis. It was noted that low concentration of BAP alone or in combination with NAA produced lower number of 1 or 2 shoots (Table 2). However, the higher concentration of BAP (3.0mg/L) with NAA (1.0mg/L) in the MS medium induced higher amount of 94.25% [Fig. 1(b&c)] callus for shoot formation followed by the same growth regulators supplemented in MS medium in different concentrations such as BAP at 2.5 mg/L, NAA at 1.0 and 2.0 and 1.0mg/L respectively induced to produce 88 and 86% of shoot formation.

Root induction

The *in vitro* derived shoots of the species were carefully removed and implanted on the MS medium containing individual or combinations of different growth regulators of IAA and BAP with NAA for root induction. The highest percentage of root formation (87.65%), greater number of roots per shoot (8.17roots/shoot) and lengthy (8.65cm) were observed in MS medium supplemented with BAP and NAA (3.0 and 1.0mg/L) (Table 3) [Fig. 1(d)]. However, the lower concentrations of BAP with NAA and IAA were not inducing roots.

Acclimatization

The plantlets were transferred to hardening medium composed by autoclaved garden soil, sand and vermicompost in the ratio of 1:1:1 by volume (Table 4) [Fig. 1(g)] which showed 72.54% of survivability.

Discussion

Callus induction

The *in vitro* regeneration protocol for *H. radicata* has been developed successfully by employing *in vitro* derived leaf explants. The highest percentage of callus response was noted in BAP and NAA at 3.0 and 0.1mg/L respectively. The synergistic effect of cytokinin in conjunction with auxin for callus formation has also been demonstrated in several medicinal plants like *Ageratum conyzoides* (Senthilkumar and Paulsamy, 2010), *Dendranthema* x *grandiflora* (Danuta, 2011) and *Exacum bicolor* (Jeeshna and Paulsamy, 2011). Many reports are already available for the enhancement of profuse callus formation by the increased in concentration of BAP supplemented MS medium (Yaghoob and Seyed, 2010; Ayyavu *et al.*, 2012; Ahlawat *et al.*, 2013).

Table 1. Effect of different concentrations of growth regulators on per cent callus induction from *in vitro* derived leaf explants of the species, *Hypochaeris radicata*

Growth regulators (mg/L)				Days required	Callus formation (%)*	Colour of the callus	Texture
BAP	NAA	2,4-D	IAA				
0.5	0.0	0.0	0.0	19	70.43±0.03^{f}	Green	Friable
1.0	0.0	0.0	0.0	18	68.34±0.56^{e}	Green	Friable
1.5	0.0	0.0	0.0	17	64.53±0.43^{e}	Green	Friable
2.0	0.0	0.0	0.0	17	59.90±0.27^{d}	Green	Friable
2.5	0.0	0.0	0.0	19	37.85±0.75^{b}	Yellowish green	Friable
3.0	0.0	0.0	0.0	19	23.95±0.22^{b}	Brown	Hard
0.5	0.5	0.0	0.0	20	70.10±0.09^{f}	Green	Friable
1.0	0.5	0.0	0.0	22	75.41±0.32^{f}	Yellow	Friable
1.5	0.5	0.0	0.0	23	82.00±0.12^{g}	Yellow	Friable
2.0	0.5	0.0	0.0	24	92.03±1.00^{h}	Yellowish green	Friable
2.5	0.5	0.0	0.0	26	91.23±0.44^{h}	Yellowish green	Friable
3.0	0.5	0.0	0.0	29	93.33±1.09^{h}	Yellowish green	Friable
0.5	1.0	0.0	0.0	30	50.34±0.04^{d}	Yellow	Friable
1.0	1.0	0.0	0.0	27	63.09±0.45^{e}	Yellow	Friable
1.5	1.0	0.0	0.0	23	74.45±0.34^{f}	Yellowish green	Friable
2.0	1.0	0.0	0.0	20	86.04±0.17^{g}	Yellowish green	Friable
2.5	1.0	0.0	0.0	19	94.98±0.56^{h}	Yellowish green	Friable
3.0	1.0	0.0	0.0	15	98.83±0.05^{h}	Yellowish green	Friable
0.0	0.0	0.2	0.0	12	96.36±0.11^{h}	Pale yellow	Friable
0.0	0.0	0.5	0.0	13	93.44±0.33^{h}	Yellow	Friable
0.0	0.0	1.0	0.0	15	72.37±0.05^{f}	Light brown	Soft
0.0	0.0	1.5	0.0	18	47.48±0.03^{c}	Light brown	Soft
0.0	0.0	2.0	0.0	21	33.29±0.22^{b}	Brown	Hard
0.0	0.0	2.5	0.0	24	20.39±0.18^{a}	Brown	Hard
0.0	0.0	3.0	0.0	27	19.04±0.42^{a}	Dark brown	Very hard
0.5	0.0	0.5	0.0	20	69.18±1.04^{e}	Green	Friable
0.5	0.0	1.0	0.0	21	58.00±0.77^{d}	Brown	Friable
0.5	0.0	1.5	0.0	22	57.05±0.37^{d}	Brown	Friable
0.5	0.0	2.0	0.0	23	52.93±0.19^{d}	Dark brown	Hard
0.5	0.0	2.5	0.0	23	49.22±0.07^{c}	Dark brown	Hard
0.5	0.0	3.0	0.0	25	35.03±0.03^{b}	Dark brown	Hard
0.0	0.5	0.0	0.0	19	78.85±0.39^{f}	Yellow	Friable
0.0	1.0	0.0	0.0	17	81.05±0.48^{g}	Yellow	Friable
0.0	1.5	0.0	0.0	15	83.74±0.33^{g}	Yellowish green	Friable
0.0	2.0	0.0	0.0	20	85.76±0.74^{g}	Yellowish green	Friable
0.0	2.5	0.0	0.0	23	73.27±0.63^{f}	Brown	Hard
0.0	3.0	0.0	0.0	25	65.78±0.93^{e}	Dark brown	Hard
0.5	0.0	0.0	0.5	20	79.03±0.45^{f}	Yellow	Friable
1.0	0.0	0.0	0.5	22	80.04±0.44^{g}	Yellowish green	Friable
1.5	0.0	0.0	0.5	21	84.20±0.52^{g}	Yellowish green	Friable
2.0	0.0	0.0	0.5	24	87.45±0.73^{g}	Yellowish green	Friable
2.5	0.0	0.0	0.5	28	87.98±0.06^{g}	Green	Friable
3.0	0.0	0.0	0.5	30	89.04±0.42^{gh}	Green	Friable

*Mean of three replicates of 30 explants ± standard deviation. Values with the column followed by the same superscript are not significantly different as determined by ANOVA at $p<0.05$.

Table 2. Effect of different concentrations of growth regulators on shoot initiation, shoot number and shoot length from *in vitro* derived callus of the species, *Hypochaeris radicata*

Growth regulators (mg/L)			Culture response (%)*	No. of shoots /callus*	Shoot length (cm)*
BAP	NAA	IAA			
0.5	0.0	0.0	09.34±0.03[a]	01.00±0.01[a]	1.03±0.04[a]
1.0	0.0	0.0	08.67±0.43[a]	01.54±0.43[a]	1.00±0.64[a]
1.5	0.0	0.0	08.08±0.12[a]	01.74±0.74[a]	1.98±0.74[a]
2.0	0.0	0.0	07.90±0.65[a]	01.32±0.19[a]	2.87±0.64[b]
2.5	0.0	0.0	09.56±0.23[a]	01.43±0.54[a]	2.99±0.53[b]
3.0	0.0	0.0	11.83±0.10[b]	02.04±0.43[b]	2.05±0.03[b]
0.5	0.5	0.0	10.01±0.18[b]	02.85±0.76[b]	2.06±0.53[b]
1.0	0.5	0.0	15.54±1.78[b]	02.46±0.51[b]	1.04±0.18[a]
1.5	0.5	0.0	23.78±0.35[c]	02.94±0.03[b]	1.02±0.32[a]
2.0	0.5	0.0	32.15±1.18[d]	03.00±0.02[c]	2.05±0.64[b]
2.5	0.5	0.0	52.10±0.22[f]	03.98±0.32[c]	2.94±0.14[b]
3.0	0.5	0.0	75.14±0.03[h]	05.43±0.65[d]	3.02±0.22[c]
0.5	1.0	0.0	20.57±0.24[c]	02.03±0.30[b]	1.64±0.53[a]
1.0	1.0	0.0	43.23±0.09[e]	03.96±0.43[c]	2.04±0.96[b]
1.5	1.0	0.0	82.45±0.45[i]	12.64±0.32[f]	3.30±0.53[c]
2.0	1.0	0.0	86.02±0.33[i]	03.32±0.22[c]	3.01±0.03[c]
2.5	1.0	0.0	88.02±0.66[i]	03.02±0.21[c]	3.04±0.21[c]
3.0	1.0	0.0	94.25±0.05[j]	07.98±0.75[e]	9.54±0.43[e]
0.0	0.5	0.0	08.85±0.35[a]	01.00±0.32[a]	2.04±0.53[b]
0.0	1.0	0.0	11.45±0.05[b]	01.93±0.64[a]	2.05±0.11[b]
0.0	1.5	0.0	13.76±0.22[b]	01.42±0.21[a]	2.01±0.53[b]
0.0	2.0	0.0	25.34±0.32[c]	01.64±0.16[a]	2.33±0.11[b]
0.0	2.5	0.0	28.27±0.63[c]	02.02±0.21[b]	3.05±0.16[c]
0.0	3.0	0.0	35.78±0.32[d]	02.43±0.04[b]	3.02±0.04[c]
0.5	0.0	0.5	38.43±0.75[d]	02.25±0.07[b]	2.02±0.14[b]
1.0	0.0	0.5	56.34±0.22[f]	03.03±0.02[c]	2.05±0.64[b]
1.5	0.0	0.5	54.31±0.75[f]	02.94±0.76[b]	2.65±0.14[b]
2.0	0.0	0.5	60.55±0.22[g]	03.07±0.29[c]	3.01±0.75[c]
2.5	0.0	0.5	60..91±0.25[g]	03.25±0.73[c]	3.90±0.18[c]
3.0	0.0	0.5	69.34±0.17[gh]	03.89±0.25[c]	4.03±0.67[d]

*Mean of three replicates of 30 explants ± standard deviation. Values with the column followed by the same superscript are not significantly different as determined by ANOVA at $p<0.05$.

Senthilkumar *et al.,* (2007) emphasized the importance of BAP with NAA for the induction of callus in the other member of Asteraceae, *Acmella calva*. The MS medium fortified with 2,4-D from 0.2 to 3mg/L showed well differentiation of callus colour sequentially pale yellow-yellow-light brown-brown-dark brown. Roy *et al.,* (2008) also found similar trend of colour change of callus in the species, *Gymnema sylvestris* by the supplementation of 2,4-D in different concentrations.

Shoot regeneration

The shoot formation was highly effective (94.25%) in the MS medium containing BAP and NAA at 3.0 and 1.0mg/L respectively. The results indicate that the BAP in combination with NAA was found to be most suitable for the better response of calli to produce shoots during subculturing. Similarly, the number of shoots produced by callus (7.98shoots/callus) and the shoot length (9.54cm) were greater on the same

prescribed medium. Dennis and Sreejesh (2004) reported that the higher amount of BAP along with lower concentration of NAA for effective shoot formation in the species, *Benincasa hispida.* Raghavendra and Sudhakar (2014) explained that the higher concentration of BAP and lower concentration of NAA will facilitate shoot proliferation is a common event in auxin-cytokinin interaction. They also described the enhanced rate of multiple shoot induction in cultures supplemented with BAP and NAA may be largely ascribed due to increased rates of cell division induced by cytokinin (BAP). Cells in this zone divide with the faster pace and thus, produced large number of shoots. This result is at par with the findings in Crape myrtle (Niranjan *et al.,* 2010) and fluted pumpkin (Olumayowa *et al.,* 2014).

Table 3. Effect of different concentrations of growth regulators on root initiation, root number and root length from *in vitro* derived shoots of the species, *Hypochaeris radicata*

Growth regulators (mg/L)			Shoots rooted (%)*	No. of roots/ shoot*	Root length (cm)*
BAP	NAA	IAA			
0.5	0.0	0.0	06.02±0.32^{b}	-	-
1.0	0.0	0.0	02.04±0.43^{a}	-	-
1.5	0.0	0.0	03.36±0.03^{a}	-	-
2.0	0.0	0.0	02.03±0.26^{a}	-	-
2.5	0.0	0.0	04.64±0.75^{a}	-	-
3.0	0.0	0.0	05.02±0.17^{b}	-	-
0.5	0.5	0.0	04.00±0.02^{a}	-	-
1.0	0.5	0.0	15.83±0.02^{c}	1.20±0.23^{a}	2.65±0.43^{c}
1.5	0.5	0.0	20.01±0.64^{d}	2.11±0.40^{b}	2.30±0.32^{c}
2.0	0.5	0.0	21.73±0.96^{d}	2.57±0.31^{b}	2.45±0.08^{c}
2.5	0.5	0.0	39.95±0.30^{g}	2.91±0.07^{b}	3.00±0.15^{d}
3.0	0.5	0.0	74.81±0.71^{m}	3.29±1.02^{c}	3.54±0.34^{d}
0.5	1.0	0.0	12.64±0.32^{c}	1.51±0.09^{a}	1.30±1.04^{b}
1.0	1.0	0.0	17.54±0.74^{d}	1.87±0.77^{a}	1.27±0.84^{b}
1.5	1.0	0.0	32.63±0.03^{f}	2.76±0.89^{b}	1.88±0.43^{b}
2.0	1.0	0.0	52.86±0.24^{i}	3.29±0.65^{c}	2.98±0.21^{c}
2.5	1.0	0.0	72.76±1.05^{m}	6.44±0.83^{e}	5.54±0.95^{e}
3.0	1.0	0.0	87.65±0.19^{n}	8.11±0.08^{f}	8.65±0.22^{f}
0.0	0.5	0.0	20.43±0.05^{d}	1.52±0.09^{a}	0.59±0.23^{a}
0.0	1.0	0.0	28.03±0.10^{e}	1.70±0.15^{a}	1.23±0.67^{b}
0.0	1.5	0.0	45.09±0.19^{h}	2.30±0.38^{b}	1.76±0.45^{b}
0.0	2.0	0.0	58.00±0.85^{j}	2.51±0.42^{b}	2.37±0.42^{c}
0.0	2.5	0.0	62.07±0.09^{k}	3.98±0.76^{c}	2.54±0.64^{c}
0.0	3.0	0.0	75.53±0.15^{l}	4.66±0.34^{d}	3.87±0.16^{d}
0.0	0.0	0.5	29.02±0.43^{e}	1.23±0.84^{a}	0.34±0.11^{a}
0.0	0.0	1.0	39.05±0.64^{g}	1.40±0.39^{a}	0.61±0.43^{a}
0.0	0.0	1.5	56.95±0.54^{j}	1.89±0.65^{a}	1.54±0.86^{b}
0.0	0.0	2.0	61.03±0.22^{k}	2.48±0.22^{b}	1.74±0.77^{b}
0.0	0.0	2.5	68.33±0.13^{l}	2.60±0.42^{b}	2.30±0.28^{c}
0.0	0.0	3.0	74.03±0.66^{m}	2.54±0.83^{b}	3.93±0.36^{d}

*Mean of three replicates of 30 explants ± standard deviation. Values with the column followed by the same superscript are not significantly different as determined by ANOVA at $p<0.05$.

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Table 4 Effect of various composition of hardening medium on survivability of plantlets of *Hypochaeris radicata*

Hardening medium composition (v/v)	No. of plantlets under hardening	No. of plantlets survived*	Survivability (%)*
Garden soil: sand: vermicompost (1:1:1)	30	23±0.09^{d}	72.54±0.02^{e}
Red soil: sand: vermicompost (1:1:1)	30	13±0.12^{c}	55.07±0.87^{d}
Vermicompost: soil (1:1:1)	30	11±0.10^{b}	44.76±0.14^{b}
Red soil: sand (1:1:1)	30	10±0.20^{a}	33.61±0.09^{a}
Decomposed coir waste: perlite: compost (1:1:1)	30	9±0.22^{c}	23.72±0.66^{c}

*Mean of three replicates of 30 explants ± standard deviation. Values with the column followed by the same superscript are not significantly different as determined by ANOVA at $p<0.05$.

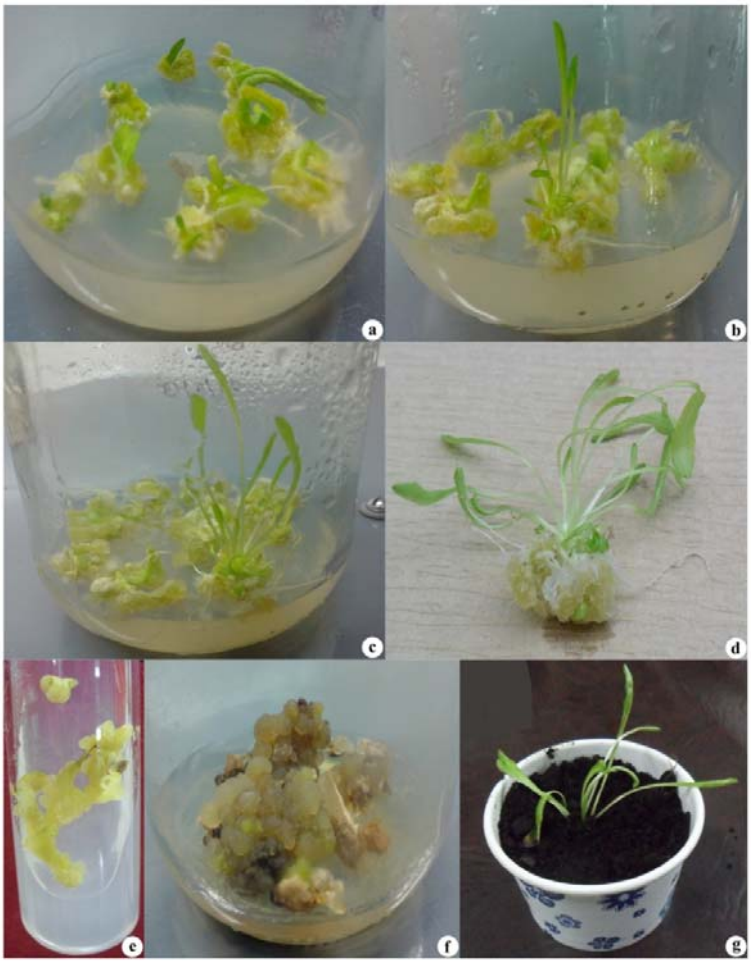

Fig. 1. Micropropagation of *Hypochaeris radicata.* A) Effective callus formation from the *in vitro* derived leaf explants in the MS medium containing BAP and NAA at 3.0 and 1.0mg/L. **B)** Successful shooting by subculturing of *in vitro* derived callus in the MS basal medium containing the BAP and NAA at 3.0 and 1.0mg/L respectively. C**)** After 4 weeks of culture. **D)** High amount of rooting was observed in the BAP and NAA at 3.0 and 1.0mg/L respectively. **E)** Callus formation in the MS medium containing 2,4-D at 0.2mg/L (Pale yellow colour with friable) **F)** Callus formation in the MS medium containing 2,4-D at 3mg/L (dark brown and hard) **G)** An acclimatized potted plantlet in the hardening medium composed by garden soil, sand, vermicompost in the ratio of 1:1:1 by volume.

Root induction and Acclimatization

The greater root induction (87.65%) was observed in the MS medium fortified with BAP and NAA at 3.0 and 1.0mg/L respectively. Similar trend of results have been reported in other Asteraceae member, *Wedelia chinensis* (Bipasha *et al.,* 2010). The auxin-cytokinin conjunction controls the

root meristem development (Werner *et al.,* 2003; Dello loio *et al.,* 2007). Several studies have shown that cytokinin and auxin mutually regulate their signaling pathways or their meristems through certain integrators, which are the basis of interaction between these two hormones to determine a specific developmental output in root meristem (Dello loio *et al.,* 2008; Moubayindi *et al.,* 2009). Rooted plantlets were transferred to pots containing garden soil: sand: vermicompost (1:1:1), which gave 72.54% survival rate. The acclimatized plants showed normal growth and were morphologically similar to the mother plants.

Conclusion

From the present study, it can be concluded that the presence of BAP and NAA is essential for callus mediated plant regeneration of *Hypochaeris radicata.* Thus, the cytokinin and auxin interact synergetically to control the balance of cell division and differentiation. And also it is particularly important to control formation and maintenance of meristems that are essential to establish the whole plant. It could be the source for somoclonal variant selection in agricultural field and also it will be useful for the conservation of wild individuals in high hills of Nilgiris and to meet the demand as well.

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