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In vitro shoot regeneration of hypocotyl explants of *Foeniculum vulgare*-a culinary and medicinal herb

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Abstract

A reproducible protocol for in vitro shoot regeneration and plantlet establishment was developed from hypocotyl-derived callus explants of Foeniculum vulgare. Callus induction frequency was the highest (92%) on SH (Schenk & Hildebrandt) medium supplemented with 4.44 µM BA (N^6-benzyladenine) and 1.34 µM NAA (α-naphthaleneacetic acid) followed by 86% and 80% on MS (Murashige & Skoog) and NN (Nitsch & Nitsch) medium respectively after 40 d of culture. The organogenic callus induced better shoot regeneration frequency (92%) on SH medium containing 6.66 µM BA as compared to 87% and 75% on MS and NN medium respectively. The highest number of 9.2 shoots per callus explant was achieved with the mean shoot length of 5.48 cm on SH medium supplemented with 6.66 µM BA followed by decreasing shoot production of 8.2 shoots and 7.3 shoots per callus explant on MS and NN medium respectively. The in vitro regenerated shoots produced the maximum root induction frequency of 94% on SH medium with 1.34 µM NAA, while on MS and NN media,frequencies of root induction were 86% and 77% respectively. There were 6.6 roots per shoot with an average root length of 6.4 cm on SH medium with a level of1.34 µM NAA, while MS and NN media with the same level of auxin (1.34 µM NAA) induced an average of 5.8 and 5.3 roots per shoot respectively after 60 d of culture. In vitro derived plantlets acclimated successfully with 100 % survival.

Keywords: Foeniculum vulgare, Callus, Hypocotyl, Regeneration

1. Introduction

The species *F. vulgare* (Fennel) is an aromatic and flavor rich perennialherb with culinary and medicinal uses. The species *Foeniculum vulgare* is with soft feathery, almost hair-like foliage found in northern temperate regions and in the tropical parts favor's warm, sunny localities. Besides, it is well established for its pharmacological properties and thus much valued as carminative, digestive and diuretic agents (4).The development of an in vitro culture system would deal exceptional opportunities for producing pharmaceuticals in the laboratory without having to depend on field cultivation (20).

. To our best knowledge, there have been no reports on successful acclimatization of this species prior to this present investigation underscoring the need for a complete reproducible protocol for rapid regeneration and plantlet recovery of this species. Thus, in this study an efficient and reproducible protocol was established from hypocotyl explants of 30 d old in vitro seedlings of *Foeniculum vulgare.*

2. Materials and Methods

2.1Expalnt source for callus induction

Seedlings of*Foeniculum vulgare* were established

as per the seed sterilization procedure followed in the earlier report of *Carum copticum* (10) after obtaining seeds from source organization namely Central Institute of Agriculture Engineering, Bhopal, India. Seeds were exposed to the treatment solution consisting of 0.1% (w/v) $HgCl_2$ for the duration of 3 min followed by washing seeds three times with sterile double distilled water ensuring removal of traces of sterilant. For germination growth, these seeds were inoculated on three culture media consisting of MS (12), NN (13) and SH (17) each containing different concentrations of gibberellic acid (GA_3: 0.75, 1.50, 3.0, 3.75 µM). In vitro seedling culture was continuously maintained for the constant source of hypocotyl explants for the present study.

2.2 Callus induction and proliferation

Hypocotyls explants (~1 cm) were prepared from in vitro raised 30 d old seedlings of *Foeniculum vulgare* and inoculated on callus induction media comprising MS, NN and SH containing plant growth regulators such as BA (1.11, 2.22, 4.44, 6.66 µM) with NAA (1.34, 2.69 µM) or Kn (1.16, µM) with NAA (1.34, µM).

The pH was measured in all the media and adjusted to 5.8 either by 1N NaOH or 1N HCl prior to addition of 0.8% (w/v) agar (Merck, India) as solidifying substance. Molten medium (15ml) was dispensed into 25 × 150 mm glass culture tubes (Borosil, India) followed by capping with aluminium foil prior to autoclaving at 121°C and 1.04 kg cm^2 for 20 minutes duration. Culture vessels were placed in the growth room under a photoperiodic length of 16h duration of light period and 8h duration of dark period at 35 µE $m^{-2}s^{-1}$ provided by white fluorescent tube lights (40 W. Philips) and relative humidity varying from 55% to 60% at 25±2°C. Callus induction (%) was recorded after 40 d of culture.

2.3 Shoot regeneration from callus culture

Callus pieces (~3-5 mm) with cut end surface in contact with MS, NN and SH media supplemented with different concentrations of BA (1.11, 2.22, 4.44, 6.66 µM), Kn (1.16, 2.32, 4.64 µM), 6-(γ-γ, dimethylallyamino purine) (2iP) (1.24, 2.45, 4.91 µM) and GA_3 (0.75, 1.50, 3.0 µM) either singly or in combination of BA (1.11, 2.22, 4.44, 6.66 µM) with Kn (1.16, 2.32 µM) or BA (1.11, 2.22, 4.44, 6.66 µM)) with 2-iP (1.24, 2.45 µM) or BA (1.11, 2.22, 4.44, 6.66 µM) with GA_3 (0.75, 1.50 µM).

Regenerated shoots were inoculated on MS, NN and SH media supplemented with different concentrations of BA (1.11, 2.22, 4.44, 6.66 µM), Kn (1.16, 2.32, 4.64 µM), 2iP (1.24, 2.45, 4.91 µM), GA_3 (0.75, 1.50, 3.0 µM) either singly or in combination of BA (1.11, 2.22, 4.44, 6.66 µM) with Kn (1.16, 2.32 µM) or BA (1.11, 2.22, 4.44, 6.66 µM)) with 2iP (1.24, 2.45 µM) or BA (1.11, 2.22, 4.44, 6.66 µM)) with GA_3 (0.75, 1.50 µM) for shoot regeneration. In vitro raised shoots were transferred to fresh medium every 20 days for further proliferation. Percentage of callus regenerating shoots, number of shoots per callus and shoot length were recorded after 60 d of culture.

2.4 Induction of rooting of in vitro raised shoots

Regenerated shoots (~2-3cm) after six weeks of culture in the shoot regeneration and proliferation medium were transferred to MS or NN or SH media containing either IAA, indole-3-butyric acid (IBA) or NAA at different concentrations such as IAA (1.42, 2.84 µM) or IBA (1.23, 2.46 µM) or NAA (1.34, 2.69 µM) for root induction. Percentage of shoot producing roots, average number of roots per shoot and root length were recorded after 60 d of culture.

2.5 Aacclimatization of rooted shoots

Regenerated rooted shoots were removed from culture vessels and washed under slowly running tap water to make the roots free from adhering medium traces prior to transfer to pots containing a mixture of 1:1 ratio of autoclaved vermicompost and soil. Pots containing plants were surrounded with polyethylene bags for duration of fourteen days for keeping humidity percentage high. Thereafter, the hardened plants were shifted to bigger pots where plants were growing healthy at medicinal garden of the institute.

2.6 Statistical Analysis

Each experiment was repeated thrice with 10-12 replicates and was completely randomized design. Data analysis was carried out by one way analysis of variance (ANOVA) and the means were scored using Tukey's test on statistical package of SPSS (ver. 20).Treatments were significantly different at $P \leq 0.05$.

3. Results

3.1 Seed germination

Successful seed germination (100%) was observed on SH medium with 0.75 µM GA_3 after 30 d while MS and NN media with higher concentration of GA_3 (1.50 µM) stimulated 100% germination (data not shown). Germinated seedlings were used as source for explants (hypocotyls) for callus induction (see Figure 3 a-c).

3.2 Callus initiation

The hypocotyl explants of *F.vulgare* were successfully induced on MS, NN and SH basal media with PGR for callus formation. The highest frequency of callus induction from hypocotyl explants (92%) on SH medium with 4.44 µM BA and 1.34 µM NAA was significant in contrast to 86% and 80% on MS and NN medium respectively and produced friable green callus after 40 d of culture (see Table 1 and Figure 3 a-c). Callus induction frequency of 52% was observed on SH medium with 2.22 µM BA and 2.69 µM NAA in contrast to 42% on MS and 44% on NN medium containing 2.22 µM BA and 2.69 µM NAA. However, the frequency of callus induction decreased to 26% on SH medium with 6.66 µM BA and 1.34 µM NAA while callus induction frequencies on MS and NN medium with the same plant growth regulators were 22% and 16% respectively. The next best callus frequency was (80%) on SH medium with 1.16 µM Kn and 1.34 µM NAA in contrast to 64% and 56% on MS and NN medium respectively (see Table 1 and Figure 3 a-c).

Table 1 Effect of plant growth regulator combinations on percent callus induction from hypocotyl explants of *Carum vulgare* after 40 d of culture on MS, NN or SH medium

Plant growth regulators (µM)	Callus induction (%)			Nature of callus
	MS	NN	SH	
Basal Medium	-	-	-	-
BA 1.11+1.34 NAA				
BA 2.22+2.69 NAA	-	-	-	-
BA 4.44+1.34 NAA				
BA 6.66+1.34 NAA	42^{f}±0.12	44^{f}±0.27	52^{e}±0.32	Green Friable/Organogenic
Kn 1.16+1.34 NAA				Green Friable/ Organogenic
	86^{b}±0.24	80^{c}±0.21	92^{a}±0.22	Compact / Non organogenic
				Green Friable / Organogenic
	22^{h}±0.14	16^{i}±0.16	26^{g}±0.24	
	64^{d}±0.23	56^{e}±0.18	80^{c}±0.12	

Data $\bar{X}$± SE collected after 40 d from three independent experiments each with 10/12 replicates (test tubes/flasks)

3.3 Shoot regeneration

Shoot regeneration responses were not observed on MS, NN and SH media tested even after 60 d of culture. Callus derived from hypocotyl explants of *F.vulgare* was successfully induced to shoot regeneration on MS, NN and SH media containing different concentrations of PGR. A small scale of 32% shoot regeneration frequency was observed on SH medium with BA at the level of 4.44 µM while the same level of PGR on NN and MS media these frequencies were 20% and 24% respectively. The highest shoot regeneration frequency of 92% resulted on SH medium containing 6.66 µM BA followed by 87% and 75% on MS and NN medium with the same level of PGR respectively after 60 d of culture. Among the different concentrations of Kn and 2-iP added individually to the basal media tested, best shoot regeneration frequencies were 38% on SH medium with 6.97 µMKn and while 58% on MS medium with 3.89 µM 2-iP. However, shoot regeneration frequency varied in different the combinations and concentrations such as: 6.66 µM BA and 2.32 µM Kn induced on the order of MS>NN>SH (56%, 44% and 25%), 4.44 µM BA and 2.46 µM 2-iP responded on the order of SH>NN>MS (24%,

18% and 16%) and 1.11 µM BA, 1.16 µM Kn and 1.24 µM 2-iP induced on the order of SH>MS>NN (42%, 40% and 27%). In this study, the highest number of 9.2 shoots per callus explant with an average shoot length of 5.48 cm were obtained on SH medium supplemented with 6.66 µM BA, whereas, 8.2 shoots and 7.3 shoots per callus were obtained on MS and NN medium having the same concentration of plant growth regulator respectively (see Figure 1 a-c, Figure 3 a-c). The effect of different concentrations of Kn and 2-iP added individually in the SH,MS and NN medium revealed thatSH medium with 4.64 µM Kn produced 4.7 shoots per callus while SH medium with 2.45 µM 2-iP could induce production of 5.6 shoots per callus. The other combination like 6.66 µM BA and 2.32 µM Kn on SH, MS and NN media respectively induced.The shoot production on the order of MS>NN>SH (5.2, 4.4 and 2.0 shoots per callus) while that of 4.44 µM BA and 2.46 µM 2-iP on SH, MS and NN media respectively induced on the order of NN>SH>MS (2.9, 2.6, 2.0 shoots per callus).See Figure 1a-c and Figure 3 a-c).

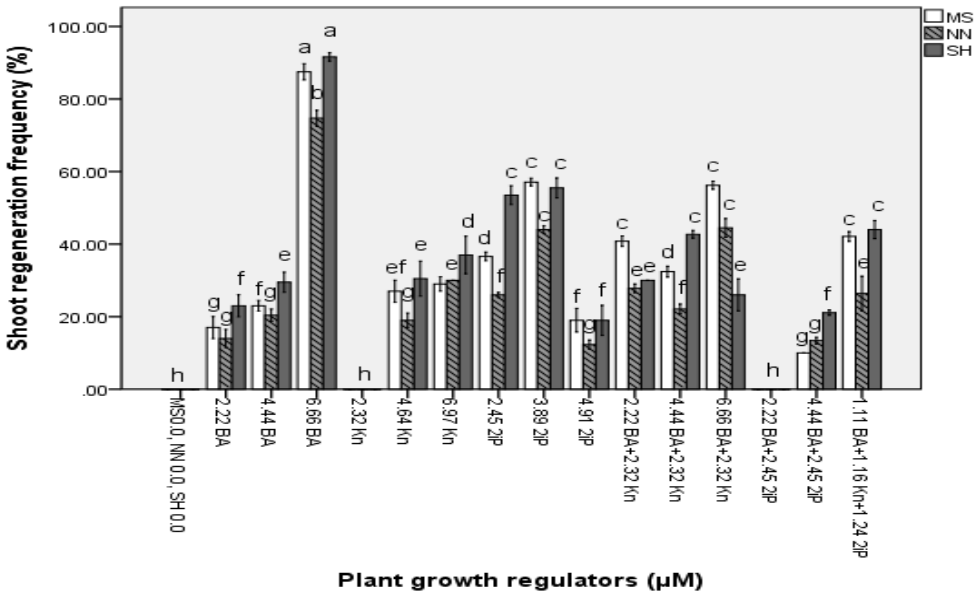


Figure 1A Influence of MS, NN and SH medium with BA, Kn or 2-iP either alone or in combination on shoot regeneration frequency (%) of C.vulgare

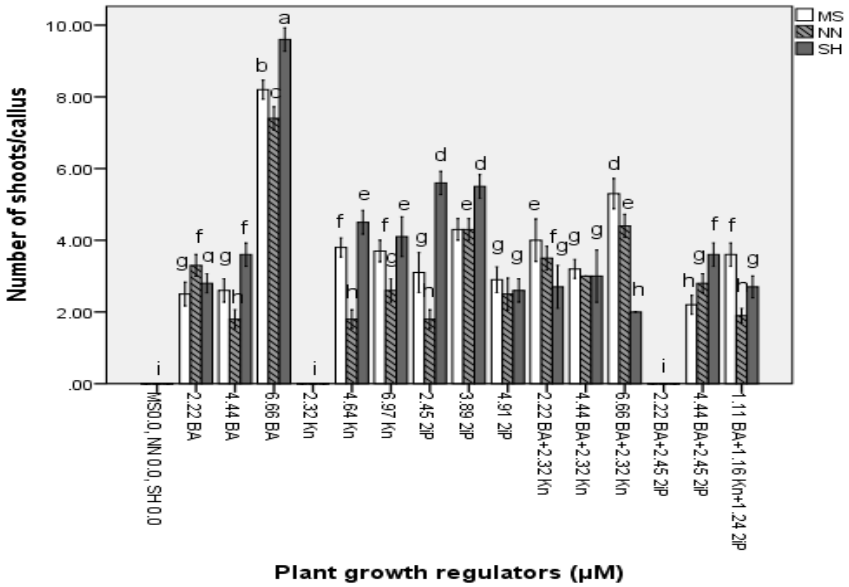


Figure 1B Effect Influence of MS, NN and SH medium with BA, Kn or 2-iP either alone or in combination on number of shoots per callus of C.*vulgare*.

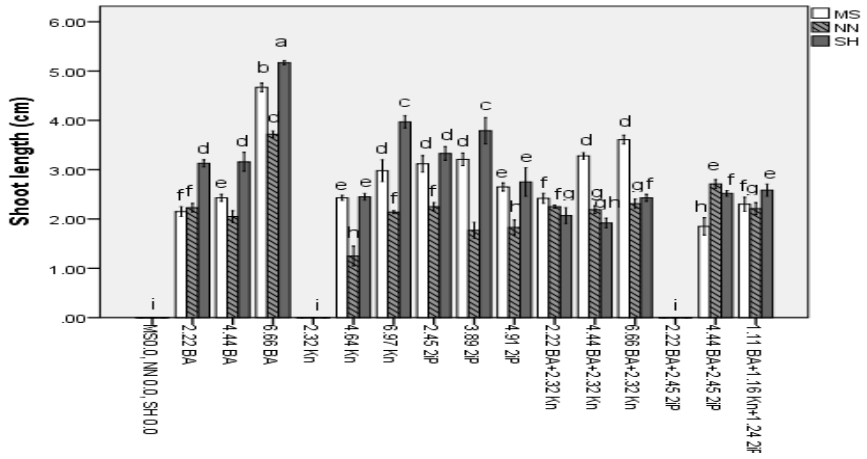


Figure 1C Influence of MS, NN and SH medium with BA, Kn or 2-iP either alone or in combination on shoot length (cm) of *C.vulgare*

3.4 Rooting of shoots

In vitro regenerated healthy shoots of *F.vulgare* did not root either on MS or NN or SH medium without auxins but rooted readily when these media were supplemented with different concentrations of auxins such as IAA or IBA or NAA. On 1.42 µM IAA supplemented SH medium with the root induction response was 50% as compared to 46% and 44% on MS and NN medium respectively whileSH medium with 1.23 µM IBA induced 40% root induction as compared to 32% and24% on MS and NN medium respectively. However, the highest root induction frequency (94%) was observed on SH medium with 1.34 µM NAA, whereas, on MS and NN medium induced 86% and 77% respectively after 60 d of culture.The highest number of 6.6 roots per shoot with an average root length of 6.4 cm were observed on SH medium with 1.34 µM NAA, while, MS and NN media with NAA (1.34 µM) induced 5.8 and 5.3 roots per shoot respectively. Moreover, NAA at 9µM on the MS, NN and SH media resulted decreased number of roots per shoot. On the other hand 1.42 µM IAA in the SH medium produced 4.8 roots per shoot with a mean root length of 4.7 cm in contrast to 3.2 roots and 2.7 roots per shoot on MS and NN medium respectively (see Table 2, Figure 3 a-c).

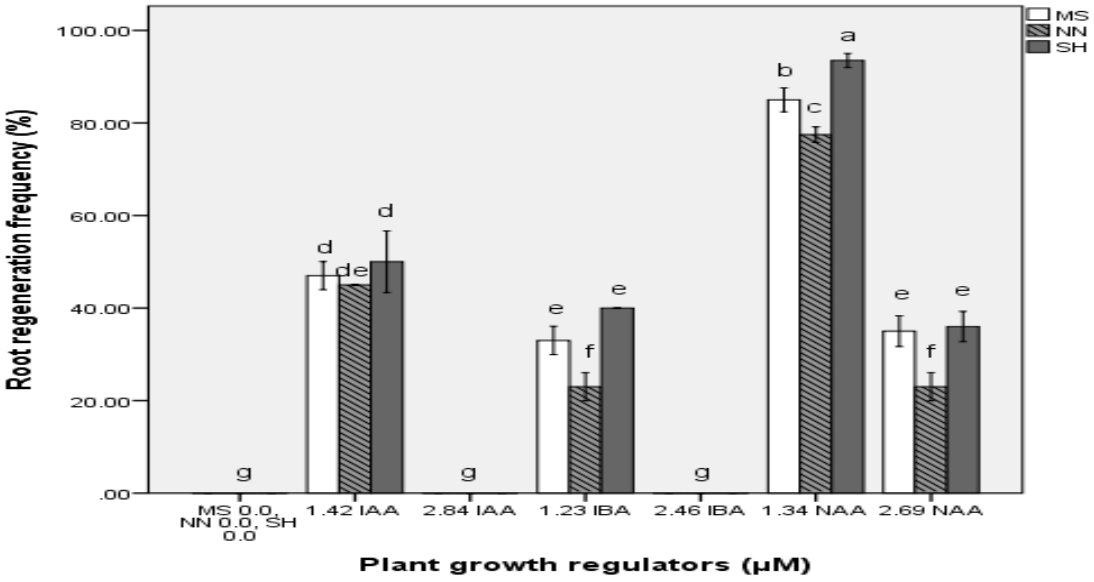


Figure 2AEffect Influence of MS, NN and SH medium with IAA, IBA or NAA on root regeneration frequency (%) of *C.vulgare*

Rooted shoots of *F.vulgare* were washed under tap water to remove adhering media from the surface of roots and then transferred to plastic pots (7x5 cm) containing a mixture of sterilized soil and vermicompost (1:1) and sheltered with polythene bag to maintain high humidity necessary for efficient acclimatization. NAA at the level of 1.34 µM was used for regular spraying to stimulate rooting and maintaining congenial environment of high humidity and kept in the growth room prior to field transfer where plants showed 100% survival rate (see Figure 3 a-c).Healthy in vitro regenerated plantlets had noapparent phenotypic variations.

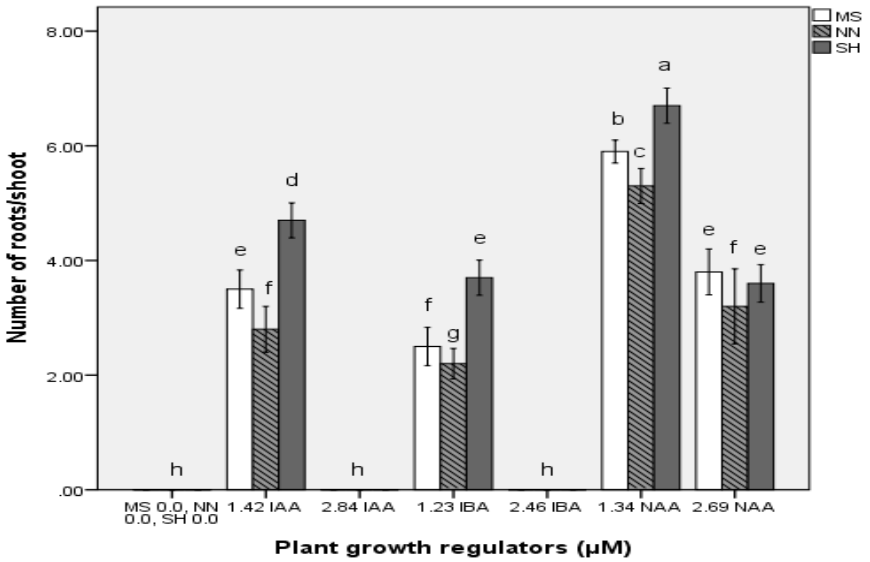


Figure 2B Effect Influence of MS, NN and SH medium with IAA, IBA or NAA on number of roots per shoot of *C.vulgare*

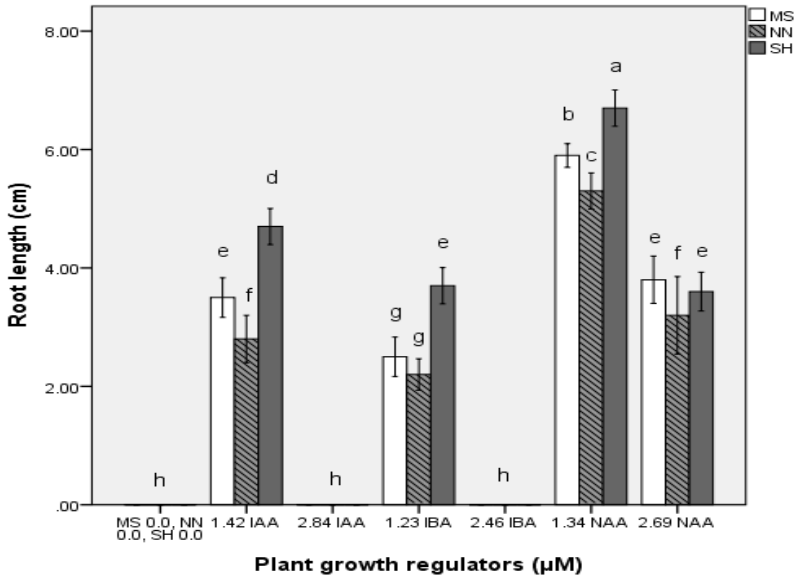
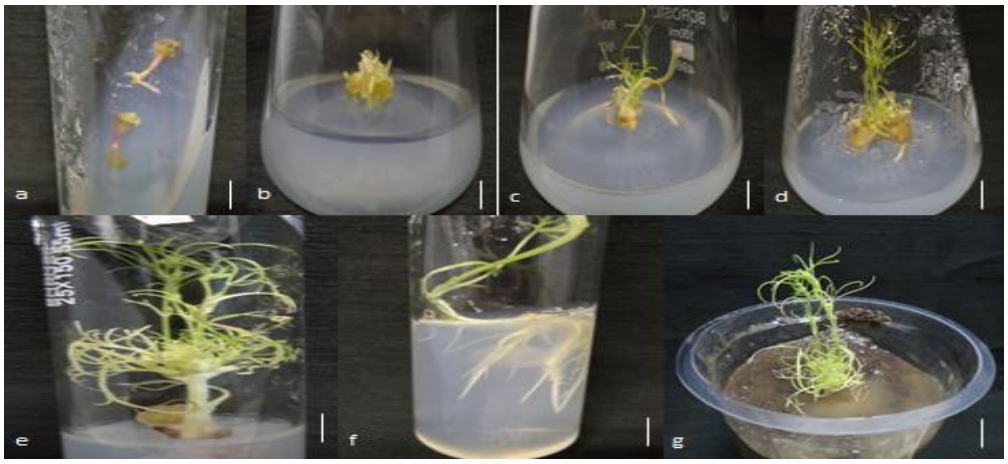


Figure 2C Influence of MS, NN and SH medium with IAA, IBA or NAA on root length (cm) of *C.vulgare.*

Figure 3AIn vitro plant regeneration of *C. vulgare* (a) germinated seed of 30 d on MS medium with 1.50 µM GA_3 (b) hypocotyl explant sourced from theseedling growing on MS medium with 6.66 µM BA, (c) shoot proliferation on MS medium with 6.66 µM BA, (d)-(e) in vitro shoot with root development on MS medium with 1.42 µM IAA, (g) A plantlet growing under field conditions, Bar(s) in (a)-(g) represents 1 cm.



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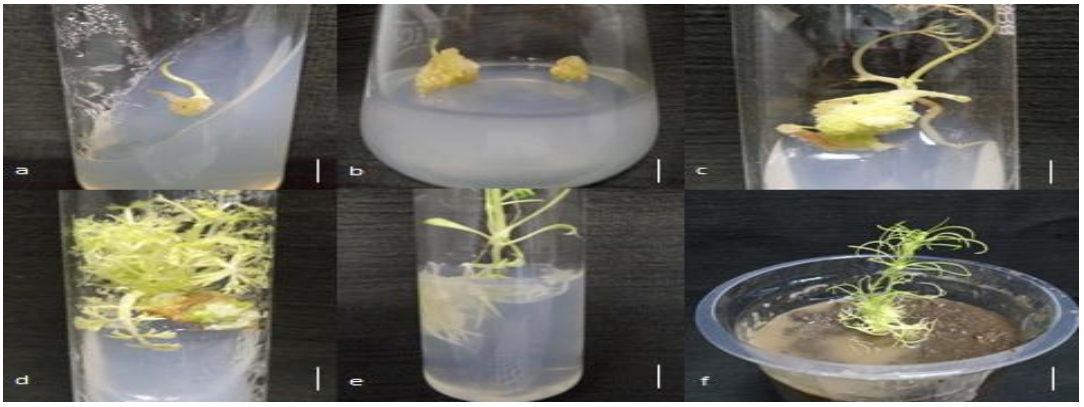

Figure 3B In vitro plant regeneration of C. vulgare (a) germinated seed of 30 d on NN medium with 1.50 µM GA3 (b) hypocotyl explant sourced from the seedling growing on NN medium with 6.66 µM BA, (c) shoot proliferation on NN medium with 6.66 µM BA, (d)-(e) in vitro plantlet with root development on NN medium with 1.44 µM IAA, (f) A plantlet growing under field conditions, Bar(s) in (a)-(f) represent 1 cm.

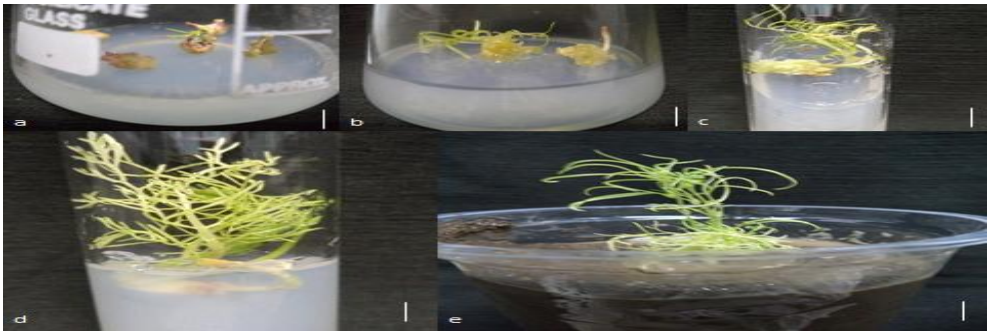

Figure 3CIn vitro plant regeneration of *C. vulgare*:(a) germinated seed of 30 d on SH medium with 0.75 µM GA_3 (b) hypocotyl explant sourced from the seedling growing on SH medium with 6.66 µM BA, (c) shoot proliferation on SH medium with 6.66 µM BA, (d) in vitro shoot with root development on SH medium with 1.44 µM IAA, (e) A plantlet growing under field conditions, Bar(s) in (a)-(e) represent 1 cm.

Discussions

The hypocotyl explants of *F.vulgare* were successfully induced on MS, NN and SH basal media with PGR for callus formation. These observations conforms to the earlier observations on callus induction from hypocotyl and radical explants of *Asparagus densiflorus* on MS medium (21), whereas in vitro cultures of fennel reported 100% callus formation on MS medium with IAA, Kn and 2-iP each at 0.5 mg/l (3). Similarly, hypocotyl and radical explants of *Asparagus densiflorus* were induced to maximum callus induction and proliferations (95%) on MS medium with 0.5 mg/l BA and 0.3 mg/l NAA (21), while in *Ammi majus* cotyledonary leaves produced callus on MS medium supplemented with 2 mg/l IAA, 0.5 mg/l Kn and 40 ml Adenine (14). In accord with the shoot regeneration from callus cultures, previous reports indicated that the most effective shoot regeneration of 88.8% from hypocotyl and cotyledon-derived callus of *Brassica oleracea* var. *capitata* seedling on MS medium with 8.88 µM BAP and 0.53 µM NAA producing the highest nuber of 7.5 shoots/buds per callus explant (5). In line with our observation it was reported that hypocotyl derived callus explants of *Foeniculum vulgare*(16) seedlings produced 100% shoot regenerating frequency with 8 shoots per explant on MS medium containing BA (0.1 mg/l) and NAA (0.1 mg/l) whilein *Ruta graveolens* (2)leaf segment-derived callus produced 70% shoot bud frequency with maximum production of 32.2 shoots per unit callus on MS medium with 7.5 µM BA but obtained enhanced shoot bud regeneration frequency of 92% with the highest number of 92.4 shoots per unit callus having a shoot length of 4.1 mm on MS medium containing 7.5 µM BA plus1.0 µM NAA. Also similar observations have been recorded in *Helicteres isora*(19) where shoot regeneration frequency of 67% occurred from nodal explant-derived callus producing 3.2 shoots per 0.5g callus on MS medium with 2.22 µM BA and 2.32 µM Kn within 35 d of culture, in*Ipomoea mauritiana*(7) optimum shoot regeneration frequency (30%) with 2-3 shoots per callus were obtained from internodal segment-derived callus on MS medium containing 1.0 mg/l BAP and 0.2 mg/l IAA while plants were successfully regenerated (over 65%) from immature leaf derived-callus in *Bambusa ventricosa* on 13.3 µM BA and 2.7 µM NAA (22).

Similar findings of root initiation were reported in many medicinal plants including *Ipomoea mauritiana* in which 6.2 roots per shoot with a root length of 4.42 cm were obtained on MS medium with 0.5 mg/l NAA (7) and 7.5 roots with an average root length of 4.16 cm produced in *Curcuma longa* on MS medium with 3.0 mg/l NAA (9). In *Brassica oleracea* var. *capitata* in vitro regenerated shoots rooted on MS medium with 5.37 µM NAA (5), while in vitro raised shoots of *Carum copticum* were induced to root formation on MS medium with 0.5 mg/L NAA (18). Similarly, in *Glycine max* rooting of in vitro regenerated shoots occurred on B5 medium containing 14.7 µM IBA (15) while in *Primula vulgaris* shoots rooted on WPM (11)medium with 0.5 mg/L IBA (6).

Plantlets were acclimatized successfully using natural soil or vermiculite and soil (1:1) in many other plant species such as in *Primula vulgaris* (6), *Momordica charantia* (1), *Ipomoea maurtiana* (7), and *Salvia officinalis* (8).

Conclusion

An efficient and reproducible protocol was established from hypocotyl explants of in vitro seedlings through indirect organogenesis of *Foeniculum vulgare*. This regeneration system showed large scale shoot regeneration from hypocotyl-derived callus and high rooting ability. These plantlets survived acclimatization and grew healthy in the medicinal garden. Moreover, hypocotyl explant-derived callus proliferation may serve as a source of medicinally important secondary metabolites. Thus, this reproducible regeneration protocol holds promise for sustainable supply of sterile plant materials to the pharmaceutical industries besides substantial contribution towards conservation of germplasm.

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References

1. Agarwal M. (2015). Tissue culture of *Momordica charantia* L.: A review. Journal of Plant Science 3(1):24-32.

2. Ahmad N, Faisal M, Anis M, Aref IM. (2010). In vitro callus induction and plant regeneration from leaf explants of *Ruta graveolens* L. South African Journal of Botany. 76: 597-600.
3. Anizidei M, Vivona L, Schiff S, Bennici A. (1996). In vitro callus culture of *Foeniculum vulgare*:callus characteristics in relation to morphologenesis. Plant Cell Tissue and Organ Culture. 45:263-268.
4. Bhattacharyya B, Johri BM. (1998). Flowering Plants - Taxonomy and Phylogeny. Springer Verlag, Berlin, Heidelberg. New York. 85-86.
5. Gerszberg A, Hnatuszko-Konka K, Kowalczyk T. (2015).In vitro regeneration of eight cultivars *Brassica oleracea* var. *Capitata*. In Vitro Cellular and Developmental Biology Plant. 51:80-87.
6. Hayta S, Smedley MA, Li J, Wendy AH, Gilmartin PM. (2016). Plant regeneration from leaf-derived callus cultures of Primrose (*Primula vulgaris*). Horticulture Science. 51(5):558-562.
7. Islam MS, Bari MA. (2013). Rapid in vitro multiplication, callogenesis and indirect shoot regeneration in *Ipomoea mauritiana*- a rare medicinal plant in Bangladesh. Journalof Medicinaland Aromatic Plant. 2:138-140.
8. Jafari S, Daneshvar MH, Salmi MS, Abaldi ALJ. 2017. Indirect organogenesis and plant regeneration in common Sage (*Salvia officinalis*): An important medicinal plant of Iran. Modern Applied Science 11(5):1-8.
9. Koarapatchaikol K, Kanmarangkol S, Kaewraksa JK. (2017). In vitro plant regeneration via callus culture in turmeric (*Curcuma longa* L.). Burapha Science Journal. 22(1):1-13.
10. Mandal J, Sharma P. (2016). In vitro micropropagation of *Carum copticum* L. Journal of Pharmacological Reports. 1:108.
11. Mc Cown BH, Lloyd G. (1981). Woody Plant Medium (WPM)-A Mineral Nutrientt Formulation for Microculture of Woody Plant Species. Horticulture Science. 16: 453.
12. Murashige T, Skoog F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiology Plant. 15:473-497.
13. Nitsch JP and Nitsch C. (1969). Experimental androgenesis in *Nicotiana*. Phytomorphology. 19:389-404.
14. Purohit M, Pande D, Dutta A, Shrivastava PA. (1995). Enhanced xanthotoxin content in regenerating cultures of *Ammi majus* and Micrpropgagation. Planta Medicine. 61pp.
15. Radhakrishnan R, Ranjithakumari BD. (2007). Callus induction and plant regeneration of Indian soybean (*Glycine max* (L.) Merr. cv. CO_3) via half seed explant culture. Journal of Agriculture and Technology.3(2):287-297.
16. Saxena SN, Kothari P, Rathore SS, Khan IU. (2012). Organogenesis in Fennel (*Foeniculum vulgare*). International Journal of Seed Spices. 2(2):1-4.
17. Schenk RU, Hildebrandt AC. (1972). Medium and techniques for induction and growth of monocotyledonous and dicotyledonous-Plant Cell Cultures. Canadian Journal of Botany. 50: 199-204.
18. Sharma P, Mandal J (2018). Organogenesis of seeding explants of C*arum copticum*. Journal of Herbs Spices and Medicinal Plants. 24(2):124-133.
19. Shriram H, Kumar V, Shitole, MG. (2008). Indirect organogenesis andmplant regeneration in *Helicteres sisora* L.- An important medicinal plant. In Vitro Cellular and Developmental Biology Plant. 44(3):186-193.
20. Sudha CG, Seeni S. (2001). Establishment and analysis of fast growing normal rootculture of *Decalpis arayalpathra*, a rare endemic medicinal plant. CurrentScience.81(4):1479-1482.
21. Toma RS, Rsheed KA.(2012). In vitro propagation through seed culture and regenerationof *Asparagus Densiflorus* L. through callus cultures derived from hypocotyls. International Journal of PureApplied Science and Technology. 9(2): 94-102.
22. Wei Q, Cao J, Qian W, Xu M, Li Z, Ding Y. (2015). Establishment of an efficientmicropropagation and callus regeneration system from the axillary buds of *Bambusaventricosa*. Plant Cell Tissueand Organ Culture. 122: 1-8.