J. Basic. Appl. Sci. Res., 8(7)26-33, 2018

ISSN 2090-4304
Journal of Basic and Applied Scientific Research
www.textroad.com

"*In vitro* propagation of the rare *Pulicaria incisa* DC. Plant"

Heba Elsayed Ghareb

Tissue Culture Unit, Department of Genetic Resources, Ecology and Dry Land Agriculture Division, Desert Research Center, 11753 El-Matareya, 1 Mathaf El-Matareya St., Cairo, Egypt

Received: September 9, 2018
Accepted: November 2, 2018

ABSTRACT

A high frequency and rapid *in vitro* propagation protocol has been established for the propagation of *Pulicaria incisa* (Lam.) DC. (Asteraceae). *P. incisa* is a very rare and multipurpose medicinal shrub native to Egypt. It is used medicinally by Egyptian Bedouins for the treatment of heart diseases and several serious diseases. The infusion of the plant was evaluated for using as a functional beverage for inhibition and treatment of brain injuries and neurodegenerative diseases. This plant is severely affected by uncontrolled overcutting and overgrazing due to its high palatability for animals. In the present study, shoot tips and stem nodal segments of *P. incisa* were taken as a source of explants. The highest percentage of survival was obtained in winter. Explants were cultured on Murashige and Skoog (MS) media supplemented with different concentrations of N^6 – (2 – isopentenyl) adenine (2iP) individually or in combination with β – naphthalene acetic acid (NAA) for the establishment and multiplication stage. MS medium supplemented with 0.25 mg l^{-1} 2iP was found to be the best treatment for growth induction of both explants. The regenerated shoots were successfully subcultured for six subculture passages with four weeks intervals. Various auxin concentrations [(NAA and indole – 3 – butyric acid (IBA)] were used for root induction. Half strength MS medium supplemented with 2 mg l^{-1} IBA was the best rooting medium. Rooted shoots of *P. incisa* were transferred to the greenhouse and showed about 70% survival. The represented *in vitro* propagation protocol for *P. incisa* offers a chance for germplasm conservation and mass production of this valuable medicinal plant.

KEYWORDS: Shaay gabali, wild tea, aromatic, tissue culture, micropropagation, conservation.

INTRODUCTION

Medicinal plants are widely distributed in many parts all over the world and useful in the treatment of many diseases and health improvement over the past hundreds of years. They are playing an important role in traditional medicine and pharmacological industries (Dakah *et al*., 2014).

Family Asteraceae, previously known as Compositae, is one of the biggest families of the flowering plants, containing approximately 1530 genus and about 23000 species. This family compresses about 10% of the world flora. Plants of family Asteraceae have been widely reported for their biological activity due to their huge chemical constituents (Chaib *et al*., 2017).

Genus *Pulicaria* contains about 80 species, distributed in North Africa, Asia and Europe. Many essential oils with useful pharmacological activities were isolated from various *Pulicaria* species (Shahat *et al*., 2017). In Iran, *Pulicaria* genus is commonly used as a medicinal plant, herbal tea and flavoring agent (Chaib *et al*., 2017).

Pulicaria incisa (Lam.) DC. sub species *incisa* is a very rare desert plant; growing in Egypt (Boulos, 2009). It is known in Arabic as "Shaay gabali" and in English as "wild tea". *P. incisa* plant has an excellent aromatic smell and reported for its essential oil. The plant infusion is consumed as a substitute of tea**.** It has been collected by Egyptian Bedouins for the treatment of heart diseases and several serious diseases in traditional medicine (Ewais *et al*., 2014).

P. incisa contains tannins, flavonoids, linoleic, oleic and palmitic acids. So, it was used in Sudan as an ingredient for manufacturing a local perfume (Abd El-Gleel and Hassanien, 2012). Biological studies on the plant revealed its antispasmodic, antimicrobial, antitumor and antioxidant activities. It contains high amounts of unsaturated fatty acids, which used in decreasing total cholesterol, total lipid and triglyceride levels. So, it has been proposed as a potential hypocholestremic agent (Elmann *et al*., 2013). The infusion of the plant was evaluated for using in the prevention of neurodegenerative diseases and for treatment of brain injuries (Elmann *et al*., 2012).

The natural propagation of *P. incisa* plant in Egyptian deserts are facing many stresses as a result of the severe environmental conditions, rainless and overexploitation of the plant resources

Corresponding Author: Heba Elsayed Ghareb, Tissue Culture Unit, Department of Genetic Resources, Ecology and Dry Land Agriculture Division, Desert Research Center, 11753 El-Matareya, 1 Mathaf El-Matareya St., Cairo, Egypt. hebaghareb_drc@yahoo.com

(uncontrolled overcutting and overgrazing) due to its high palatability for animals which led to the rare production of the plant seeds (Shaltout *et al*., 2010).

Tissue culture is an effective technique used for *in vitro* propagation of plants, which are difficult to propagate naturally, production of disease free plants, somatic hybridization, improve the genetic make-up of commercial plants and production of haploid plants. Also, it is played a vital role in secondary metabolites production, napthoquinones, pigments, shikonin, sweeteners, flavors, natural colorants and pharmaceuticals. Moreover, plant tissue culture is used in research for biochemists, geneticists, plant breeders, plant pathologists and other researchers (Gaurav *et al*., 2018).

Hence, this study is aimed to establish an efficient plant propagation protocol through direct organogenesis from shoot tips and stem nodal segments of *P. incisa*. To the best of our knowledge, this is the first report on the micropropagation of the wild *P. incisa* plant.

MATERIALS AND METHODS

This research was carried out in the Plant Tissue Culture Unit in the Department of Plant Genetic Resources, Desert Research Center.

1. Plant material and sterilization

Plant specimens were collected from wild plants of *P. incisa* (Figure 2a) grown in Saint Catherine Protectorate, South Sinai, Egypt and identified by Dr. Ibrahim Abdelrafee Elgamal, Nature Conservation Sector, Egyptian Environmental Affairs Agency, South Sinai, Egypt. Shoot tips and stem nodal segments were used as explants. The Explants were washed under running tap water for 30 min. Shoot tips were sterilized by soaking in 1% sodium hypochlorite solution for 25 min., whereas stem nodal segments were sterilized by soaking in 2% sodium hypochlorite solution for 30 min. Some drops of Tween 20 (w/v) were added to both explants during sterilization. Finally, all explants were washed five times with sterilized distilled water. Explants were implanted vertically on a sterilized nutrient medium.

2. Culture medium and conditions

Shoot tip and stem nodal segment explants, about 3 cm long, were cultured on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with 3% sucrose, 100 mg l^{-1}myo-inositol and gelled with 3 g l^{-1}phytagel. Different plant growth regulators (PGRs); auxins [β – naphthalene acetic acid (NAA) and indole – 3 – butyric acid (IBA)] and cytokinin [N^6 – (2 – isopentenyl) adenine (2iP)], at different concentrations, were added individually or in combination to the medium to evaluate the effect of PGRs on shoot establishment, multiplication and rooting. PGRs free MS medium was used as control. The pH of the medium was adjusted to 5.7 – 5.8 before autoclaving at 121°C under a pressure of 1.1 $kgcm^{-2}$ for 15 min. All cultures were incubated in a culture room at a temperature of 26 ± 2°C with a 16 h photoperiod under cool white fluorescent tubes of 2 k lux light intensity.

3. Effect of season on survival and browning

Plant specimens of *P. incisa* were collected in autumn, winter and spring and cultured on PGRs free MS medium for four weeks to determine the best season of plant collection for micropropagation. The survival and browning percentages were recorded for shoot tips and stem nodal segments.

4. Establishment stage

For the establishment of *P. incisa*, shoot tips and stem nodal segments were cultured on MS medium supplemented with 2iP individually at concentrations of 0.1, 0.25 and 0.5 mg l^{-1} or in combination with 0.1 mg l^{-1} NAA. PGRs free MS medium was used as control. Growth induction percentage (%), the mean number of shoots/explant, mean length of shoots (cm) and callusing percentage (%) were recorded after four weeks of culturing.

5. Multiplication stage

The established shoots were subcultured every four weeks onto the best medium of the establishment stage (MS medium supplemented with 0.25 mg l^{-1} 2iP). The mean number and length of shoots were recorded at each passage of subculturing onto the fresh growth medium up to the 8^{th} subculture.

6. Root induction

Multiplied shoots were transferred to half strength MS medium supplemented with different concentrations (0.25, 0.5, 1.0 and 2.0) of auxins (IBA and NAA), added individually for *in vitro* root

induction. Half strength MS medium without PGRs was used as control. The percentage of rooted shoots (rooting %), mean number of roots/shoot and mean root length (cm) were recorded after six weeks.

7. Acclimatization

Rooted shoots were removed from the culture medium and washed in sterilized distilled water. Then they were transferred to plastic pots containing a sterilized mixture of sand and soil (1:1 v/v), covered with a plastic cap in the greenhouse. The plantlets were irrigated regularly. The plastic caps were gradually opened within four weeks to complete acclimatization of the plants.

8. Experimental design and statistical analysis of data

All experiments were carried out by a completely randomized design. Experiments were repeated twice and the treatments had at least 15 replicates. For statistical analysis, analysis of variance of the data was carried out using ANOVA program. The differences among means for all treatments were tested for significance at 5% level using Duncan's multiple range test (Duncan, 1955). Means followed by the same letter are not significantly different at $P \leq 0.05$.

RESULTS AND DISCUSSION

1. Effect of season on survival and browning in *P. incisa* plant

Browning is mainly a result of the release of phenolics from the cutting ends of the explants. It is a natural defense mechanism in plants in response to pathogen invasion that shows the phytotoxic effect. The produced oxidized compounds may not only negatively affect the explant development, but also may cause its death (Das and Rahman, 2016).

In the present study, shoot tip and stem nodal segment explants of *P. incisa* were collected during three different seasons (autumn, winter and spring).

It is clear from Table 1 that the two explants gave different responses according to the season of collection. Spring gave significantly the lowest survival percentage and the highest percentage of browning for both types of explants. While winter gave the highest values of survival percentage for both types of cultured explants. The highest percentage of survival was 62.8 and 77.4% for shoot tips and stem nodal segments collected in winter, respectively. Martini and Papafotiou (2013) observed during the *in vitro* propagation of the rare wild *Malosorbus florentina* that the browning of the explants was the highest in March and April and declined after June. While in winter, the release of phenolics was completely not observed. The browning of the explants was affected by the season of collection for many plants, such as in apple shoot tips (Wang *et al*., 1994; Modgil *et al*., 1999 and Dobranszki *et al*., 2000).

Also, it is observed from data in Table 1 that the survival percentage was higher for stem nodal segments, compared to shoot tips during all tested seasons. While, browning percentage during different seasons were higher in shoot tips than stem nodal segments. Similar to this observation, shoot tip explants of 'Koroneiki' olive trees and *Malosorbus florentina* exhibited higher browning rates and total phenol and polyphenol oxidase activity compared with nodal explants (Martini and Papafotiou, 2013).

It can be concluded that the best time to collect the plant specimens of *P. incisa* for *in vitro* culture was during winter to obtain the optimum percentage of survival.

Table 1. Effect of seasonal variations on the survival and browning of *Pulicaria incisa* cultured *in vitro* on MS medium after four weeks.

Season	Months of collection	Explant type			
		Shoot tip		Stem nodal segment	
		Survival (%)	Browning (%)	Survival (%)	Browning (%)
Autumn	October-November	35.0^{b}	65.0^{b}	49.6^{b}	50.4^{b}
Winter	January-February	62.8^{a}	37.2^{c}	77.4^{a}	22.6^{c}
Spring	March-April	20.7^{c}	79.3^{a}	34.6^{c}	65.4^{a}

2. Establishment and multiplication

MS medium supplemented with various concentrations of 2iP (0.1, 0.25 and 0.5 mg l^{-1}) individually or in combination with 0.1 mg l^{-1} NAA were used for the establishment of *P. incisa* shoot tip and stem nodal segment explants.

Data in Table 2 reveal that MS medium supplemented with 0.25 mg l^{-1} 2iP was the best medium for the establishment of *P. incisa* shoot tips (Figure 2b). It gave the highest percentage of growth induction (83.4%), the highest value of the mean number of shoots (10 shoots/explant) and the highest mean length of shoots/explant (5.4 cm). The percentage of growth induction was maximum and insignificantly different in three media; PGRs free MS medium and media supplemented with 0.1

or 0.25 mg l^{-1} 2iP. Minimum percentage of callusing formed at the cut surfaces of the cultured explants was obtained in the same three media. Excluding the control MS medium, the medium supplemented with 0.5 mg l^{-1} 2iP or 0.1 NAA plus different concentrations of 2iP gave the lowest number of shoots/explant and the maximum amount of callus formed at the cut surfaces of explants (Table 2).

Table 2. Effect of PGRs (2iP and NAA) in MS medium on the establishment of *Pulicaria incisa* shoot tips cultured *in vitro* after four weeks.

Growth regulators conc. (mg l^{-1})		Growth induction (%)	Mean number of shoots/ explant	Mean shoot length (cm)	Callusing (%)
2iP	NAA				
0.00	**0.0**	82.0^{a}	2.7^{e}	4.0^{b}	23.0^{f}
0.10	**0.0**	83.0^{a}	8.5^{b}	4.6^{ab}	29.5^{e}
0.25	**0.0**	83.4^{a}	10.0^{a}	5.4^{a}	30.0^{e}
0.50	**0.0**	60.4^{b}	7.2^{bc}	4.5^{ab}	43.0^{d}
0.10	**0.1**	59.1^{b}	6.7^{cd}	4.3^{ab}	73.0^{c}
0.25	**0.1**	59.1^{b}	4.0^{de}	3.1^{c}	81.4^{b}
0.50	**0.1**	58.3^{b}	2.0^{e}	2.9^{c}	92.3^{a}

Table 3 clears that media supplemented with 2iP individually at any concentration gave the highest growth induction percentages and they were insignificantly different. As in shoot tip explants; MS medium supplemented with 0.25 mg l^{-1} 2iP individually was the best medium for the establishment of *P. incisa* stem nodal segment (Figure 2c). This medium gave 89.5% of growth induction, 7.3 shoots/explant and 5.3 cm mean length of shoot (Table 3). Increasing the concentration of 2iP (individually), increased the mean shoot number and length until reaching the concentration of 0.25 mg l^{-1} then they decreased at 0.5 mg l^{-1}. The same observation was noticed for the number of shoots in the presence of NAA in the culture medium.

The second best medium was MS medium supplemented with 0.1 mg l^{-1} 2iP which gave 88.0% of growth induction, 6.0 shoots/explant and 5.0 cm mean length of shoot.

Also, by increasing the 2iP concentration either individually or in the presence of NAA, the amount of undesirable callus formed at the explants cut surface was increased.

Table 3. Effect of PGRs (2iP and NAA) in MS medium on shoot establishment of *Pulicaria incisa* stem nodal segments cultured *in vitro* after four weeks.

Growth regulators conc. (mg l^{-1})		Growth induction (%)	Mean number of shoots/ explant	Mean shoot length (cm)	Callusing (%)
2iP	NAA				
0.00	**0.0**	88.5^{a}	4.3^{bc}	3.9^{b}	10.4^{g}
0.10	**0.0**	88.0^{a}	6.0^{ab}	5.0^{a}	20.0^{f}
0.25	**0.0**	89.5^{a}	7.3^{a}	5.3^{a}	32.0^{e}
0.50	**0.0**	89.0^{a}	3.3^{c}	3.6^{bc}	52.1^{d}
0.10	**0.1**	77.0^{b}	3.0^{c}	3.5^{bc}	66.3^{c}
0.25	**0.1**	75.0^{b}	2.7^{c}	3.0^{d}	76.6^{b}
0.50	**0.1**	73.6^{b}	2.0^{c}	3.3^{cd}	85.3^{a}

From Tables 2 and 3, it can be concluded that for the establishment of *P. incisa* plant using shoot tips or stem nodal segments; MS medium supplemented with 0.25 mg l^{-1} 2iP was the optimum treatment with low callusing percentage. The formation of callus may be a result of high endogenous hormonal levels in the plant. Therefore, the establishment of *P. incisa* needs only low concentrations of 2iP without adding auxin, even at low concentrations.

The same results were presented by Jin *et al*. (2014), who obtained a high number and length of shoots per explant on MS medium containing low concentrations of cytokinin (0.1–0.2 mg l^{-1} BA) during the direct regeneration of the medicinal plant *Pogostemon cablin*. They found that adding auxin (NAA) even at low concentrations resulted in slower shoot development and growth with the formation of callus at the cut surfaces as compared to cytokinin alone.

Similarly, Rout *et al*. (2008) observed that the addition of auxin into the cytokinin containing medium decreased not only shoot multiplication but also led to the production of compact callus at the cut surfaces of the *Acacia chundra* explants.

Also, similar observations were found in *Pterocarpus santalinus* (Lakshmi *et al*., 1992), *Acacia auriculiformis* (Mittal *et al*., 1989) and *Acacia mangium* (Nanda *et al*., 2004).

For further multiplication, the established shoots of *P. incisa* were transferred to the best medium for the establishment of both shoot tips and stem nodal segments (MS medium supplemented with 0.25 mg l^{-1} 2iP). Multiplication of *P. incisa* yielded a large number of shoots (Figure 2d) through eight subculture passages at four weeks interval. The survival percentage gave 100% during all subculture passages. The number of shoots increased after each subculture and showed no sign of

decline until the 6th subculture passage. After that; the number of multiple shoots began to decrease with increasing subcultures. In the 6th subculture, the highest mean number of shoots reached 13.6 (Figure 1). In this concept; Lodha *et al*. (2015) successfully multiplied the endangered medicinal shrub, *Cadaba fruticosa* by culturing the established shoots in the same medium used in the establishment stage.

Re-culturing the established shoots to a fresh medium is a successful way to produce new shoots in a short time by renewing the juvenility of dormant meristematic cells (Sanchez *et al*., 1997). This practice was successfully employed in a number of arid plant species (Rathore *et al*., 2005; Lodha *et al*., 2014 and Patel *et al*., 2014).

Yadav and Singh (2012) reported that the rate of shoot multiplication of the medicinal plant *Glycyrrhiza glabra* depended on the number of subcultures and the average number of shoots per explant increased with each successive subculture cycle. A similar observation was also documented in *Aegle marmelos* and *Rauvolfia serpentina* (Ajithkumar and Seeni, 1998 and Alatar, 2015).

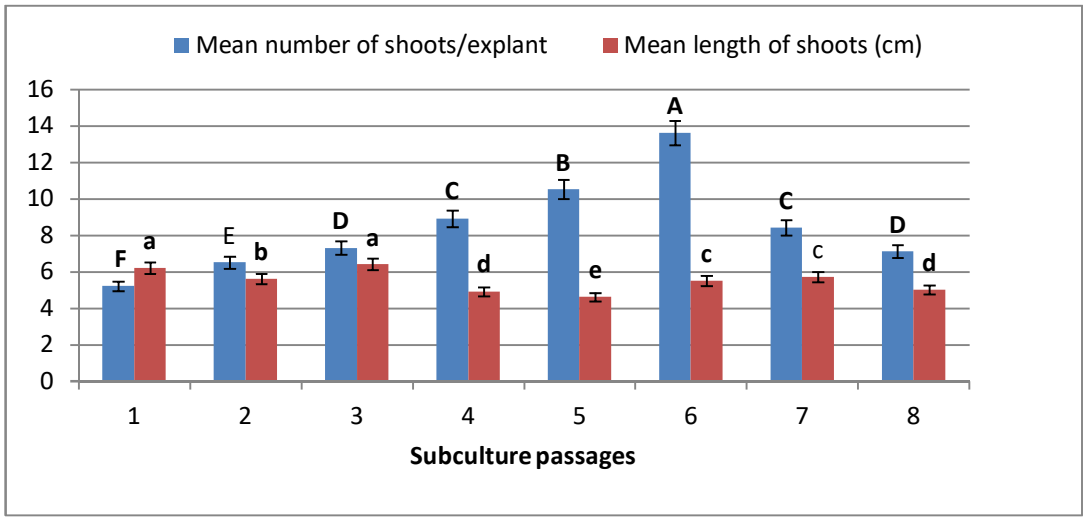

Figure 1. Effect of subculture passages on the mean number and length of the multiplied shoots of *Pulicaria incisa* on MS medium supplemented with 0.25 mg l^{-1} 2iP for four weeks.

3. Rooting and acclimatization

There are evidences that the endogenous and exogenous auxins trigger rooting in the plants during a sexual propagation (Siddiqui and Hussain, 2007; Kumar *et al*., 2009 and Babu *et al*., 2018). Different synthetic auxins like NAA and IBA are commonly used to promote root development in *in vitro* propagation. Auxin triggers the hydrolysis of the starch and the mobilization of nutrients and sugars at the base ends of the cuttings during the regeneration of adventitious roots (Babu *et al*., 2018).

Data in Table 4 reveal that addition of different concentrations of IBA or NAA to half strength MS medium led to the increase in the rooting percentage than the control medium (1/2 MS medium without PGRs). The increase in the concentration of both tested auxins increased the rooting percentage and mean number and mean length of roots. IBA was more effective in root induction than NAA. Maximum rooting percentage (80.2%), maximum mean number of roots (26.8 roots/explant) and the highest mean length of roots (14.9 cm) were achieved when explants were cultured on MS medium supplemented with 2 mg l^{-1} IBA (Figure 2e). Sixty nine percent of rooting was obtained when explants were cultured on MS medium supplemented with 1 mg l^{-1} IBA, which considered the second best medium. Also, it gave the next best number of roots (18.5 roots/explant) and the next highest length of root (13.6 cm).

The superiority of IBA in promoting root development is supported by Kumar *et al*. (2018), who developed ninety percent rooting of *Vitex negundo* (an important medicinal plant), on half strength MS medium supplemented with 0.75 mg l^{-1} IBA. Also, Reshi *et al*. (2017) found that shoots of *Anisochilus carnosus* rooted on MS medium supplemented with 2 mg l^{-1} IBA.

Rooted shoots of *P. incisa* were transferred to the greenhouse (Figure 2f). About 70% of the acclimatized plantlets were survived after four weeks of transferring into a mixture of sand: soil (1: 1 v/v). All of the transplants showed normal development.

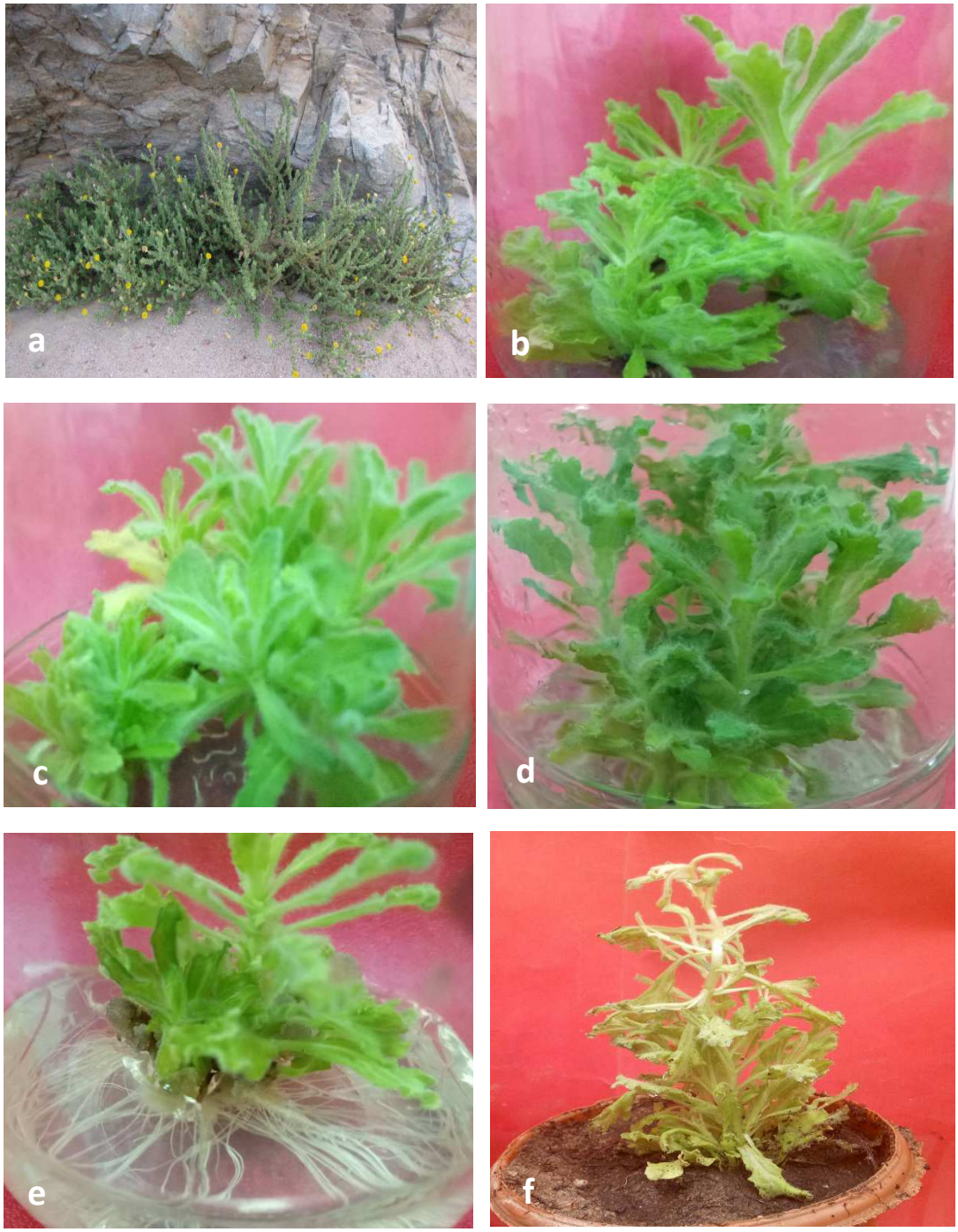

Figure 2. Micropropagation of *Pulicaria incisa*; a. *P. incisa* grown in Saint Catherine, Sinai; b. Shoot tip establishment; c. Stem nodal segment establishment; d. Multiplied shoots after six passages of subculturing; e. *In vitro* rooting; f. *In vitro* derived plantlet after four weeks of acclimatization.

Table 4. Effect of 1/2 MS medium supplemented with different concentrations of IBA or NAA on the rooting of *Pulicaria incisa* shoots after six weeks.

Growth regulators conc. (mgl^{-1})		Rooting (%)	Mean number of roots/shoot	Mean root length (cm)
IBA	NAA			
0.00	**0.00**	10.3^{e}	3.9^{cd}	7.5^{c}
0.25	**0.00**	45.0^{c}	8.3^{cd}	11.6^{b}
0.50	**0.00**	53.3^{c}	11.3^{c}	13.1^{ab}
1.00	**0.00**	69.0^{b}	18.5^{b}	13.6^{ab}
2.00	**0.00**	80.2^{a}	26.8^{a}	14.9^{a}
0.00	**0.25**	21.7^{d}	1.3^{d}	1.6^{e}
0.00	**0.50**	26.7^{d}	1.8^{d}	2.8^{e}
0.00	**1.00**	36.7^{cd}	3.0^{cd}	3.0^{e}
0.00	**2.00**	40.0^{cd}	9.0^{cd}	5.25^{d}

In conclusion, an *in vitro* propagation protocol discussed here can be applied for large-scale production of *P. incisa* plants using shoot tip and stem nodal segment explants. Winter season is the most suitable for the specimens collection. MS medium supplemented with 0.25 mg l^{-1} 2iP is optimum for the establishment and multiplication of this plant. Half strength MS basal medium supplemented with 2 mg l^{-1} IBA is the best for root induction.

Acknowledgment

The author is thankful to Prof. Dr. Ghada A. Hegazi, Head of Tissue Culture Unit, Department of Genetic Resources, Desert Research Center, for her effort in reviewing this paper.

REFERENCES

Abd El-Gleel, W. and Hassanien, M.F.R. (2012). Antioxidant properties and lipid profile of *Diplotaxis harra*, *Pulicaria incisa* and *Avicennia marina.* Acta Aliment., 41(2): 143–151.

Ajithkumar, D. and Seeni, S. (1998). Rapid clonal multiplication through *in vitro* axillary shoot proliferation of *Aegle marmelos* (L.) Corr., a medicinal tree. Plant Cell Rep., 17: 422–426.

Alatar, A.A. (2015). Thidiazuron induced efficient *in vitro* multiplication and *ex vitro* conservation of *Rauvolfia serpentina* – a potent anti-hypertensive drug producing plant. Biotechnol. Biotechnol. Equip., 29(3): 489–497.

Babu, B.H., Larkin, A. and Kumar, H. (2018). Effect of plant growth regulators on rooting behavior of stem cuttings of *Terminalia chebula* (Retz.). Int. J. Curr. Microbiol. App. Sci., 7(8): 2475–2482.

Boulos, L. (2009). Flora of Egypt Cheklist: Alhadara Publishing, Egypt.

Chaib, F., Allali, H., Bennaceur, M. and Flaminic, G. (2017). Chemical composition and antimicrobial activity of essential oils from the aerial parts of *Asteriscus graveolens* (FORSSK.)LESS. and *Pulicaria incisa* (LAM.) DC.: Two Asteraceae herbs growing wild in the Hoggar. Chem. Biodiversity, 14, e1700092.

Dakah, A., Zaid, S., Suleiman, M., Abbas, S. and Wink, M. (2014). *In vitro* propagation of the medicinal plant *Ziziphora tenuior* L. and evaluation of its antioxidant activity. Saudi. J. Biol. Sci., 21: 317–323.

Das, D.K. and Rahman, A. (2016). Phenolic compositions of Litchi shoot tips and zygotic embryos collected in different months and their effects on the explant browning and its control. Advances in Anthropology, 6: 73–92.

Dobranszki, J., Abdul-Kader, A., Magyar-Tabori, K., Jambor-Benczur, E., Buban, T., Szalai, J. and Lazanyi, J. (2000). *In vitro* shoot multiplication of apple: Comparative response of three rootstocks to cytokinins and auxin. Int. J. Hort. Sci., 6: 76–78.

Duncan, B.D. (1955). Multiple range and multiple F tests. Biometrics, 11: 1–42.

Elmann, A., Telerman, A., Mordechay, S., Erlank, H. and Ofir, R. (2012). Antioxidant and astroprotective effects of a *Pulicaria incisa* infusion. Oxid. Med. Cell. Longev., Article ID 157598.

Elmann, A., Telerman, A., Erlank, H., Mordechay, S., Rindner, M., Ofir, R. and Kashman, Y. (2013). Protective and antioxidant effects of a chalconoid from *Pulicaria incisa* on brain astrocytes. Oxid. Med. Cell. Longev., Article ID 694398.

Ewais, E.A., Abd El-Maboud, M.M. and Haggag, M.I. (2014). Studies on chemical constituents and biological activity of *Pulicaria incisa* subsp. Incisa (Asteraceae). Report and Opinion, 6(9): 27–33.

Gaurav, N., Komal, Juyal, P., Tyagi, M., Chauhan, N. and Kumar, A. (2018). A review on *in vitro* propagation of medicinal plants. J. Pharmacogn. Phytochem., 7(6): 2228–2231.

Jin, H., Deng, C.Z. and He, H. (2014). Effect of explant types and plant growth regulators on direct regeneration in medicinal plant *Pogostemon cablin*. Plant Omics Journal, 7(5): 322-327.

Kumar, A.B.S., Lakshman, K., Jayaveera, K.N., Vamshi Krishna, N., Manjunath, M., Suresh, M.V., Shivatej, Reddy, H. and Naik, S. (2009). Estimation of rutin and quercetin in *Terminalia chebula* by HPLC. Asian J. Research. Chem., 2(4): 388–389.

Kumar, A., Fayaz da M., Bhat, M.H. and Jain, A.K. (2018). An efficient protocol for *in vitro* regeneration of *Vitex negundo* an important medicinal plant. Biosci. Biotech. Res. Comm., 11(2): 256-262.

Lakshmi, S.G., Sreenatha, K.S. and Sujata, S. (1992). Plantlet production from shoot tip cultures of red sandalwood (*Pterocarpus santalinus* L.). Curr. Sci., 62: 532–535.

Lodha, D., Rathore, N., Kataria, V. and Shekhawat, N.S. (2014). *In vitro* propagation of female *Ephedra foliate* Boiss. & Kotschy ex Boiss.: an endemic and threatened Gymnosperm of the Thar Desert. Physiol. Mol. Biol. Plant, 20: 375–383.

Lodha, D., Patel, A.K. and Shekhawat, N.S. (2015). A high frequency *in vitro* multiplication, micromorphological studies and *ex vitro* rooting of *Cadaba fruticosa* (L.) Druce (Bahuguni): a multipurpose endangered medicinal shrub. Physiol. Mol. Biol. Plants, 21(3): 407–415.

Martini, A.N. and Papafotiou, M. (2013). Season and explant origin affect phenolic content, browning of explants and micropropagation of ×*Malosorbs florentina* (Zucc) Browicz. Hortscience, 48(1): 102–107.

Mittal, A., Agarwal, R. and Gupta, S.C. (1989). *In vitro* development of plantlets from axillary buds of *Acacia auriculiformis*– a leguminous tree. Plant Cell, Tissue Organ Cult., 19: 65–70.

Modgil, M., Sharma, D.R. and Bhardwaj, S.V. (1999). Micropropagation of apple cv. Tydeman Early Worcester. Sci. Hort., 81: 179–188.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant., 15: 473– 479.

Nanda, R.M., Das, P. and Rout, G.R. (2004). *In vitro* clonal propagation of *Acacia mangium* Willd. and its evaluation of genetic stability through RAPD marker. Ann. For. Sci., 61: 381–386.

Patel, A.K., Phulwaria, M., Rai, M.K., Gupta, A.K., Shekhawat, S. and Shekhawat, N.S. (2014). *In vitro* propagation and *ex vitro* rooting of *Caralluma edulis* (Edgew.) Benth. & Hook. f.: an endemic and endangered edible plant species of the Thar Desert. Sci. Hortic., 165: 175–180.

Rathore, J.S., Rathore, M.S. and Shekhawat, N.S. (2005). Micropropagation of *Maerua oblongifolia* - a liana of arid areas. Phytomorphology, 55: 241–247.

Reshi, N.A., Sudarshana, M.S. and Girish, H.V. (2017). *In vitro* Micropropagation of *Anisochiluscarnosus* (L.)Wall. Journal of J Appl. Pharm. Sci., 7 (7): 98–102.

Rout, G.R., Senapati, S.K. and Aparajeta, S. (2008). Micropropagation of *Acacia chundra* (Roxb.) DC. Hort. Sci., 35(1): 22–26.

Sanchez, M.C., San-Jose, M.C., Ferro, E., Ballester, A. and Vieitez, A.M. (1997). Improving micropropagation conditions for adult-phase shoots of chestnut. J. Hortic. Sci., 72: 433–443.

Shahat, E.A., Bakr, R.O, Eldahshan, O.A. and Ayoub, N.A. (2017). Chemical composition and biological activities of the essential oil from leaves and flowers of *Pulicaria incisa* sub. *candolleana* (Family Asteraceae). Chem. Biodiversity, 14, e1600156.

Shaltout, K.H., Sheded, M.G. and Salem, A.I. (2010). Vegetation spatial heterogeneity in a hyper arid biosphere reserve area in North Africa. Acta Bot. Croat., 69(1): 31– 46.

Siddiqui, M.I. and Hussain, S.A. (2007). Effect of indole butyric acid and types of cuttings on root initiation of *Ficus hawaii*. Sarhad J. Agric., 23(4): 919–925.

Wang, Q.C., Tang, H.R. Quan, Y. and Zhou, G.G. (1994). Phenol induced browning and establishment of shoot-tip explants of 'Fuji' apple and 'Jinhua' pear cultured *in vitro*. J. Hort. Sci., 69: 833–839.

Yadav, K. and Singh, N. (2012). Factors influencing *in vitro* plant regeneration of Liquorice (*Glycyrrhiza glabra* L.). Iran. J. Biotechnol., 10(3): 161–167.