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Research Article

IN VITRO CULTURE OF *PETROSELINUM CRISPUM* L. (PARSLEY) - AN AROMATIC HERB

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ABSTRACT

Petroselinum crispum very popular herb known as Parsely is used in culinary, pharmaceutical, perfume and cosmetics industries. It is also known for medicinal values. Although this plant has been used as model system for the production of secondary metabolites, there are scarce reports on *in-vitro* culturing of *Petroselinum crispum*. Therefore, we tried to establish a protocol for in vitro culture of *Petroselinum crispum*. Among the two media tested, SH media was found to be more efficient in callus induction and organogenesis. Among the various explants tested, node and internode was found to be best responding explants. Callus induction and shoot formation was observed in SH medium having only BAP (0.5 mg/L) and SH medium with NAA (1 mg/L) in combination with BAP (1.5 mg/L).

Keywords: *B5 Media, SH Media, 2, 4-D, NAA, BAP, Kinetin, Organogenesis*

INTRODUCTION

Petroselinum hortense (P. Miller) Nymann ex. A.W. Hill. (Syn *P. crispum Mill),* is a member of the Apiaceae family, which is commonly known as Parsley, Cilantro, Salsa and Salsa pea, Ajmood. *Petroselinum crispum* is a native of Sardinia and is extensively cultivated in the Mediterranean region and the USA. In India, the var. *crispum* is grown in limited areas in Himachal Pradesh, Punjab, Haryana, and Uttar Pradesh and in the high-altitude areas of southern India. *Petroselinum crispum* is hardy, aromatic, biennial, umbelliferous, much-branched green herb, producing a rosette of finely-divided radical leaves in the first year and a flowering stalk, up to 100 cm high in the second year. The flowers are yellow to yellowish-green, borne in compound umbels. The fruit commonly known as seeds and it has 2-3 mm long, crescent-shaped, conspicuously ridged and, consisting of mericarps. Its pleasant aroma, characteristic flavor and spicy nature are due to the presence of volatile oil. Parsley produced in the dehydrated form is mostly consumed in almost every country, since it is one of the most popular herbs known worldwide.

Petroselinum crispum has been employed in the food, pharmaceutical, perfume and cosmetics industries (Lopez *et al.,* 1999). In traditional medicine, it is considered to be a diuretic, uterine stimulant, sedative emollient and antiparasitic agent and it is commonly employed for the treatment of chronic bronchitis, bronchial asthma, and dyspepsia. Its leaves and stems are used in cystitis, edema, kidney stone, prostatitis, (Yarnell, 2002; Yanardag *et al.,* 2003; Wright *et al.,* 2007). The constituents of *Petroselinum crispum*, which include ascorbic acid, carotenoids, flavonoids, coumarins, myristicin, apiole, various ternenoic compounds, phenyl propanoids, phthalides, furano coumarins and tocopherol have been chemically investigated (Tunali *et al.,* 1999; Yanardag *et al.,* 2003). In vitro cultures of *Petroselinum crispum* have been used as model systems to study the production of secondary metabolites (Kreuzaler and Hahlbrock, 1973). Although the usefulness of *Petroselinum crispum* in medicine and culinary is well known but, there are hardly any reports on *in vitro* culture of *Petroselinum crispum.* Therefore, the aim and objective of this study was to standardize the best medium, explants, effectiveness of auxins and cytokinins and to record the morphogenetic response and to establish a protocol for regeneration.

MATERIALS AND METHODS

Plant material

Mature plant was the source of explants. The explants were young leaves base, nodes and internodes. The nodes, internodes and leaf base were excised from field grown mature plants of *Petroselinum crispum* and were washed under running tap water. Further treatments were carried out under laminar air flow. The explants were surface sterilized with 0.1% $HgCl_2$ for 5 minutes, rinsed thoroughly 4X with autoclaved distilled water, to remove traces of $HgCl_2$. The explants were cultured on B5 and SH medium supplemented with varying concentration of auxins and cytokinins.

In –vitro culture

Standardization of media and explants type:

Two different culture media such as B5 (Gamborg *et al.,* 1968) and SH (Schenk and Hildebrandt, 1972) were used in the present study to standardize the best medium, the best responding explants and type of response on a particular medium.

For standardization of media, different combination of auxins and cytokinins were used. The media consisted of full strength B5 (Gamborg *et al.,* 1968) and SH (Schenk and Hildbrandt, 1972) media. The media were supplemented with varying concentration of 2-4 Dichlorophenoxyacetic acid (2,4-D), α Naphthalene acetic acid (NAA) in the range of 0.5 mg/L, 1mg/L, 1.5mg/L and 2 mg/L; in combination with varying concentration of BAP and kinetin (0.5mg/L, 1mg/L, 1.5mg/L and 2 mg/L). The cultures were incubated and maintained at 25± 2°c under 16-h/8 h of photoperiod in culture room. The days of response, type of response and nature of the callus was recorded in regular interval.

Maintenance of callus:

Maintenance of callus growth was carried by sub culturing after every 15 days in the media which gave best results.

RESULTS AND DISCUSSION

In the present study different media and various growth-hormone combinations were used to obtain best response of the suitable explants like nodes, internodes and leaf base.

Effect of B5 media supplemented with auxins (2, 4-D, and NAA) in combination with cytokinins (Kinetin and BAP):

The response of different explants on B5 media supplemented with varying concentration of 2, 4-D in combination with varying concentration of Kinetin and BAP was studied in order to find best responding explants and morphogenesis response. It was observed that combination of higher concentration of auxin (2, 4-D) and cytokinins (kinetin) were able to induce callus (1 mg/L of 2, 4-D + 1.5 mg/L of kinetin (Fig. 1 a); 1.5 mg/L of 2, 4-D + 2 mg/L of kinetin (Fig. 1 b); only 2 mg/L of 2, 4-D).

Figure 1 a: B5 medium with 1 mg/L of 2, 4 D and 1.5 mg/L of Kinetin (nodes)
Figure 1 b: B5 medium with 1.5 mg/L of 2, 4 D and 2 mg/L of Kinetin (internode)

The callus was pale yellow-off white in colour, friable, and growth rate of callus was slow. Moreover, it was observed that, nodal explants responded early (7 days) whereas internodal explants responded late (10-13 days). However, B5 media supplemented with varying concentration of 2, 4-D in combination with varying concentration of BAP was able to induce not only callus but also organogenesis (root). It was observed that combination of higher concentration of BAP and lower concentration of auxin induced callus and rhizogenesis (only 0.5 mg/L of BAP; 0.5 mg/L of 2,4-D+1 mg/L of BAP; 1 mg/L of 2,4-D + 1.5 mg/L of BAP; 1.5 mg/L of 2,4-D + 2 mg/L of BAP). Node, internode and leaf base all showed callus induction and organogenesis, but the response was delayed (14 days). The callus was pale yellow to off white and friable. The results are presented in Table 1.

Table 1: Effect of B5 media supplemented with different concentration of 2, 4-D in combination with BAP on different explants

S. No.	Concentration mg/ l (2,4-D+ BAP)	Type of Explants	% of response	Day of induction	Types of response
1	0+0.5	Node	100%	14	Callus induction
		Internode	100%	14	Callus induction+ Rhizogenesis
		Leaf base	100%		Enlargement
2	0.5+1	Node	100%	14	Callus induction
		Internode	100%	14	Callus induction + Rhizogenesis
		Leaf base	100%	14	Callus induction
3	1+1.5	Node	100%	14	Callus induction
		Internode	Nil		Nil
		Leaf base	100%	14	Callus induction + Rhizogenesis
4	1.5+2	Node	100%	14	Callus induction + Rhizogenesis
		Internode	80%	14	Callus induction + Rhizogenesis
		Leaf base	Nil		Nil
5	2+0	Node	Nil		Nil
		Internode	Nil		Nil
		Leaf base	40%	20	Callus induction

Similarly, the effect of B5 media fortified with various concentration of NAA in combination with varying concentration of Kinetin and BAP was also studied. All the three explants cultured on B5 media supplemented with NAA or BAP/kinetin alone or combination of NAA with BAP and kinetin showed callus induction irrespective of the concentration of growth regulators used in the study (0.5 mg/L of NAA with 1 mg/L of kinetin; 1 mg/L of NAA with 1.5 mg/L of kinetin and 1.5 mg/L of NAA with 2 mg/L of kinetin; 0.5 mg/L of BAP alone; 0.5 mg/L of NAA+1 mg/L of BAP; 1 mg/L of NAA + 1.5 mg/L of BAP; 1.5 mg/L of NAA + 2 mg/L of BAP; 2 mg/L of NAA). The calli was found to be off white and friable.

Effect of SH media supplemented with auxins (2, 4-D, and NAA) in combination with cytokinins (Kinetin and BAP):

The effect of SH media supplemented with 2, 4-D, and NAA in combination with kinetin and BAP, on callus induction and organogenesis was also studied on three types of explants of *P. crispum*. It was noted that SH media supplemented with 2, 4-D and kinetin was able to induce callus and organogenesis (root). The concentration that was effective in inducing callus and organogenesis in leaf base and internodes was

0.5 mg/L of 2,4-D with 1 mg/L of kinetin (Fig. 2 c); 1 mg/L of 2,4-D with 1.5 mg/L of kinetin, and only 2 mg/L of 2,4-D (Fig. 2 d).

Fig 2 c: SH medium with 0.5 mg/L of 2, 4 D and 1 mg/L of Kinetin (rhizogenesis and callus induction in leaf base)
Fig 2 d: SH medium with 2 mg/L of 2, 4 D alone (rhizogenesis and callus induction in internode)

The callus induction and organogenesis (root) was observed within 6-8 days. The calli was found to be friable, pale yellow and fast growing. The results are presented in Table 2.

Table 2: Effect of SH media supplemented with different concentrations of 2, 4-D in combination with Kinetin on different explants

S. No.	Concentration mg/ l (2, 4-D + Kin)	Type of Explants	% response	Days of induction	Types of response
1	0+0.5	Node	80%		Enlargement
		Internode	80%		Enlargement
		Leaf base	80%		Enlargement
2	0.5+1	Node	80%		Enlargement
		Internode	60%	6	Callus induction
		Leaf base	100%	8	Callus induction + organogenesis
3	1+1.5	Node	80%		Enlargement
		Internode	100%	6	Callus induction + organogenesis
		Leaf base	100%		Swelling
4	1.5+2	Node	100%		Enlargement
		Internode	60%	6	Callus induction
		Leaf base	100%		Enlargement
5	2+0	Node	Nil		Nil
		Internode	100%	6	Callus induction + organogenesis
		Leaf base	80%	7	Callus induction + organogenesis

The combination of 2, 4-D and BAP in SH media was also found to be quite encouraging. The SH media with 0.5 mg/L of BAP alone initially formed callus in node within 6-8 days, which after one month of sub-culturing developed shoots (Fig. 3 e). Also in the same media (SH) high concentration of auxin (2,4-D) and cytokinin (BAP) i.e. 1.5 mg/L of 2 ,4-D with 2 mg/L of BAP, induced not only callus but also rhizogenesis in nodes and leaf bases explants within 13 days from days of inoculation. The results are presented in Table 3.

Table 3: Effect of SH media supplemented with different concentrations of 2, 4-D in combination with BAP on different explants

S. No.	Concentration mg/ l (2, 4-D + BAP)	Type of Explants	% response	Day of induction	Types of response
1	0+0.5	Node	100%	8	Callus induction+ Shoot formation
		Internode	100%	13	Callus induction
		Leaf base	60%	13	Callus induction
2	0.5+1	Node	100%	13	Callus induction
		Internode	100%	13	Callus induction
		Leaf base	100%	13	Callus induction
3	1+1.5	Node	100%	13	Callus induction
		Internode	100%	13	Callus induction
		Leaf base	100%	13	Callus induction
4	1.5+2	Node	100%	13	Callus induction + Rhizogenesis
		Internode	100%	13	Callus induction
		Leaf base	100%	13	Callus induction + Rhizogenesis
5	2+0	Node	100%	13	Callus induction
		Internode	100%		Swelling, No induction of callus
		Leaf base	20%		Curling, No induction of callus

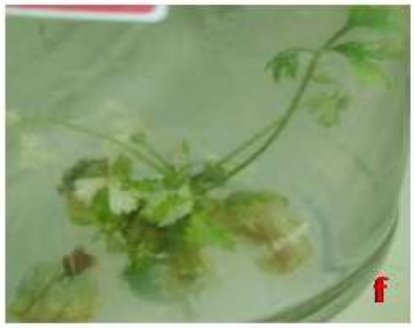

Figure 3 e: SH medium with 0.5 mg/L of BAP alone (Callus induction and shoot regeneration in nodes)
Figure 3 f: SH medium with 0.5 mg/L of BAP alone (Callus induction and shoot regeneration in internodes)

The varying combination of NAA and kinetin in SH media was not found to be so effective in inducing callus. Callus induction was observed only in SH media with 1mg/L of NAA and 1.5 mg/L of kinetin, in nodes and internodes within 12-13 days and the callus was slow growing. However, the best results were obtained with SH media having combination of NAA and BAP. The combination not only showed callus induction but also rhizogenesis and shoot formation. SH media supplemented with only 0.5 mg/L of BAP, initially induced callus in nodes, internodes and leaf base within 11-15 days. After one month of sub-culturing, the internodal explants inoculated in the SH media supplemented with 0.5 mg/L of BAP showed proliferation of callus as well as shoot formation (Fig. 3 f).

Similarly, intermodal explants in SH media supplemented with 1 mg/L of NAA and 1.5mg/L of BAP, showed proliferation of callus as well as shoot formation. Leaf base explants responded in the form of rhizogenesis in SH media supplemented with 2 mg/L of NAA alone. The calli were green in colour. The results are presented in Table 4.

Table 4: Effect of SH media on different explants supplemented with different concentrations of NAA in combination with BAP

S. No.	Concentration mg/ l (NAA+ BAP)	Type of Explants	% response	Day of induction	Types of response
1	0+0.5	Node	100%	11	Callus induction
		Internode	100%	11	Callus induction + Shoot formation
		Leaf base	100%	15	Callus induction
2	0.5+1	Node	80%	15	Callus induction
		Internode	100%	20	Callus induction
		Leaf base	100%	11	Callus induction
3	1+1.5	Node	100%	20	Callus induction
		Internode	100%	20	Callus induction + Shoot formation
		Leaf base	100%	20	Callus induction
4	1.5+2	Node	100%	20	Callus induction
		Internode	80%	15	Callus induction
		Leaf base	100%	15	Callus induction
5	2+0	Node	100%	20	Callus induction
		Internode	60%	20	Callus induction
		Leaf base	20%	20	Only Rhizogenesis

DISCUSSION

In present investigation, significant effect of media has been observed on callus induction and regeneration of *Petroselinum crispum.* B5 media with the concentration of 0.5 mg/L of 2, 4-D +1 mg/L of BAP; 1 mg/L 2, 4-D + 1.5 mg/L of BAP; 1.5 mg/L 2, 4-D + 2 mg /L of BAP and B5 media with only 0.5 mg//L of BAP could induced callus and organogenesis. In our present study, the SH medium, with the combination of 0.5 mg/L of 2, 4-D + 1 mg/L of kinetin; 1 mg/L of 2, 4-D + 1.5 mg/L kinetin; 2 mg/L of 2, 4-D alone showed callus induction and organogenesis. Also the SH medium with the combination of 1.5 mg/L of 2, 4-D + 2 mg/L of BAP induced callus and organogenesis (root) in *Petroselinum crispum.* Similarly, the SH medium with 2 mg/L of NAA alone also resulted in callus and organogenesis (root). However, the best response was obtained with nodal and the internodal explants cultured in SH media supplemented alone with 0.5 mg/L of BAP and 1 mg/L of NAA in combination with 1.5mg/L of BAP which showed proliferation of callus as well as shoot formation.

Auxin and cytokinin when used alone or in combination with each other have an effect on callus induction and organogenesis which is well documented in various plant parts. The effect of auxin 2, 4-D and NAA on callogenesis have been reported by many workers on different plants (Malik *et al.,* 2003, Tahir *et al.,* 2011). The promotive effect of auxin and cytokinin interaction on callus proliferation has been reported by many workers in plants (Neibour *et al.,* 2008; Gopitha *et al.,* 2010). Generally a high concentration of auxin with a low concentration of cytokinin has been recommended for initiation and

proliferation of callus. In *Daucas carota* though various parts were found to be totipotent to regenerate the nodal explants and stem explants has been considered to be the appropriate explants for inducing the multiple shoot in the present investigation.

In present investigation, when the concentration of BAP was increased to 0.5 to 1.5 mg/L, shoot formation was observed. Hence, increase in concentration of BAP has positive effect on the shoot formation which also reduced the day of response. Sripichit *et al.,* (1987) observed that BAP was more effective than kinetin in inducing shoot formation on MS medium. In the present investigation, the SH media supplemented with only BAP (0.5 mg/L), the nodal explants initiated the shoot formation. Joshi *et al.,* (2003) also reported that MS medium supplemented with BAP (1mg/L) + NAA (0.5mg/L) was the ideal condition for the induction of micro-shoot from the nodal segment of *Foeniculum vulgare* a member of Apiaceae.

From all above observation it can be concluded that among the two media tested, SH media was found to be more efficient in callus induction, organogenesis. Among the various explants tested node and internode was found to be best responding explants. SH medium, alone with BAP (0.5 mg/L) could not only induce callus formation but shoot formation. Similarly, SH medium supplemented with NAA (1 mg/L) in combination with BAP (1.5 mg/L) induced callus and shoot formation.

From this study we could standardized the protocol for in vitro culture of *Petroselinum crispum*. This protocol will be helpful for rapid and large scale production. These results are expected to be used for future work such as cellular studies on in vitro plants, effect of radiation in vitro flowering and some molecular aspect such as protein content etc can be conducted with this protocol. Apart from that, protoplast isolation and transformation work in *Petroselinum crispum* can be done to identify another aspect in *Petroselinum crispum.*

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