# *IN VITRO* PROPAGATION OF CARROT *(DAUCUS CAROTA)* L.

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**Abstract:** *In vitro* method for rapid propagation of Daucus *carota* L. was developed. Root, stem, leaf and nodal explants were cultured on MS medium supplemented with different concentration and combinations of hormones. Multiple shoot were induced from the nodal explant of *D. carota* by culturing them in MS medium supplemented with 0.5 mg/l of 6-benzylaminopurine (BAP). Differentiation of shoot initiated after one week of culture, and after eight weeks of primary culture, an average of six plantlets were developed from a single shoot. The nodal explant also induced same number of multiple shoot in MS medium supplemented with 2 mg/l of BAP and 1 mg/l of α-nephthalene acetic acid (NAA). The shoots when sub-cultured in the medium supplemented with 1 mg/l NAA produced roots after five weeks of sub-culture.

Root and leaf explants induced roots and callus when cultured on MS medium with NAA at the rate of 1 mg/l and 2 mg/ l. Similarly, stem explants also induced roots and callus in the same concentration of hormones whereas few multiple shoot were induced when cultured on MS supplemented with 2 mg/l of BAP and 1 mg/l of NAA. This result suggests that this methodology can be applied for the rapid and mass propagation of this species.

**Key words:** *In vitro; Propagation*; MS medium; Explant; Culture.

## INTRODUCTION

Family Apiaceae is one of the largest families of angiosperm comprising 300 genera and 2,500 –3,000 species. The member of the family, mostly temperate herbs, can be found in northern temperate regions and in tropical highlands located throughout the world. This family is characterized by its distinctive umbrella-like inflorescence, the umbel. Carrot (*Daucus carota)* L. is an important member of family Apiaceae that was well known to Greeks and Romans for medicinal purposes (Kochhar, 1998). Only in recent time, it has been well known human food and is cultivated all over the world as a popular vegetable (Anonymous, 1948). The carrot is a cold-season crop, growing best in a deep, moist, loose, well-drained, light loam soil. It is cultivated between 1200-2700 m altitudes. The plant is a heavy feeder, especially of potash.

Carrot is valued as food mainly because it is rich source of the fat-soluble hydrocarbon, carotene ($C_{40}H_{56}$) the β form of which is the precursor of Vitamin A. The modified root of carrot is the most widely used part as food but all parts of carrot plant are equally valuable. Carrot roots are used as vegetables for soups, stews, curries and pies, grated roots are used as salad, tender roots are pickled. Carrot juice is a rich source of carotene; it is used for coloring butter and other food articles. Besides the food value, different parts of carrot can be used for different medicinal purposes. Carrot roots are used as refrigerant and seeds as aromatic, stimulant and carminative. They are useful in the kidney diseases, in dropsy, nervine tonic, aphrodisiac and given in uterine pain. An infusion of carrot has long been used as a folk remedy of threadworms. Carrot increases the quantity of urine and large amount of carrot to the diet has a favorable effect on the nitrogen balance.

Normally carrot is propagated from seeds; however it has been a good experimental material for *in vitro* culture. Somatic embryogenesis in carrot has already been reported (Kamada *et al.,* 1989; Liu *et al.*, 1994). The present investigation was carried out to develop a protocol for the *in vitro* multiplication of carrot.

## MATERIALS AND METHODS

The seeds (Fig. 1) of *D. carota* were used for this experiment, which were bought from the local market of Kathmandu. For seed culture, the healthy seeds were washed with detergent water and were kept in running water for one hour. They were washed with distilled water for 3-5 times and surface sterilized by immersing them in the solution of sodium hypochlorite (1%) for 15 min, 70% ethanol for 1 min and finally by rinsing three times with sterile water.

### Seed and explant culture

Seeds were cultured on MS medium supplemented with 3% sucrose and solidified with 0.8 % agar. pH of the medium was adjusted to 5.8 before autoclaving. Cultures were maintained at $25^0C \pm 2^0C$ and exposed to 16 hours light per day.

Seeds were germinated after two weeks of culture with normal roots, shoots and leaves. For explant culture, root, stem, leaf and nodal parts from the *in vitro* grown seedlings were aseptically excised in 3 mm pieces and cultured on MS medium (Murashige and Skoog, 1962) supplemented with different concentrations of NAA (0, 0.5 1.0, and 2.0 mg/l) and BAP (0,

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0.5, 1.0, 2.0 mg/l), either alone or in combination. Morphogenetic response was examined at the given cultured condition. Cultures were subcultured into fresh media once every six weeks. The regenerated plantlets were transferred to pots in order to grow into normal plants.

## RESULTS

### Seed germination

The seeds of *D. carota* were cultured on MS medium, which germinated after three days of inoculation. All the plantlets developed with normal roots and shoots. The plantlets (Fig. 2) measured about 14 cm height having 4 cm long root after three weeks of culture. The over all germination percentages of the seeds were 79%.

### Root, leaf, node and stem culture

The root explant obtained from *in vitro* culture of seeds, when cultured on MS + BAP (0.5 mg/l), formed green callus after four weeks of culture. On MS + NAA (0.5 mg/l), explants induced greenish callus after three weeks of culture. When the concentration of NAA was increased to 1 mg/l, roots were produced after three weeks of culture (Fig 3), which started to form callus after four weeks. Further increase in the

**Fig 1:** Seeds of *Daucus carota.*

**Fig 2:** Seedling of *D. carota* grown in MS medium (3 weeks old).

**Fig 3:** 4 week old roots and callus formation from root explant of *D. carota* on MS + NAA (1 mg/l).

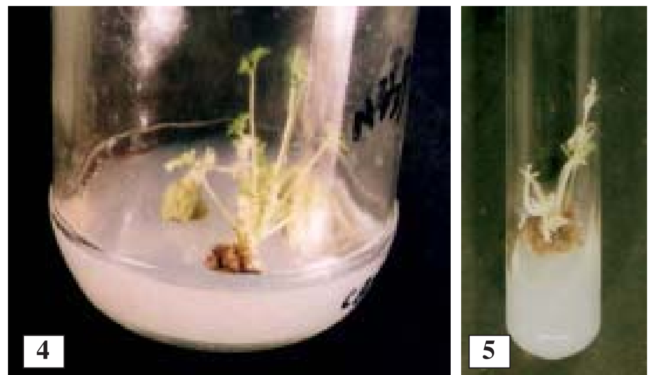


**Fig 4:** 10 week old multiple shoots proliferated from nodal explant of *D. carota* on MS + BAP (0.5 mg/l).

**Fig 5:** 9 week old multiple shoots proliferated from callus from nodal explant of *D. carota* on MS + BAP (2 mg/l) + NAA (1 mg/l).

concentration of NAA to 2 mg/l, roots appeared after two weeks of culture from which yellowish callus was induced after four weeks. On equal combination of NAA and BAP (0.5, 1 and 2 mg/l), the root segments swelled up and the cut ends gave callus, which showed no further responses.

The leaf segments obtained from *in vitro* grown seedling were cultured either on MS medium alone or supplemented with BAP (0.5, 1 and 2 mg/l) and NAA (0.5 mg/l). In MS media supplemented with NAA 1 mg/l and 2 mg/l, small roots on the upper surface of the explant appeared after three weeks of culture which exhibited very slow growth and from the lower surface of the explant callus was developed which was yellow in colour.

When the nodal explants were inoculated in hormone free MS medium and combination with NAA (1 mg/l and 2 mg/l), plantlets were developed. The explant cultured on MS media supplemented with BAP (1 mg/l), shoots were induced after three weeks of culture while in BAP 2 mg/l, shoots were induced after one week of culture. The nodal explant cultured on MS supplemented with BAP (0.5 mg/l) initiated a shoot after one week of culture and started giving multiple shoots after four weeks of culture In this culture condition, an average of 6 shoots were differentiated from each node by eight weeks of culture (Fig 4). Same number of multiple shoots were also induced from the callus obtained from the nodal explant when cultured on MS in combination with BAP (2 mg/l) and NAA (1 mg/l) (Fig 5).

The stem explants obtained from the *in vitro* germinated seedlings showed great totipotency in producing the callus in MS medium combined with BAP plus NAA or BAP alone in different concentration and combition. On MS medium along with NAA 1 mg/l and 2 mg/l, the stem explants initiated the formation of roots after three weeks of culture, which increased in length greatly and produced the callus after 4 weeks of culture (Fig 6). From this callus, multiple shoots were induced after 5 weeks of culture (Fig 7).

## DISCUSSION

*D. carota* is one of the important food-valued as well as medicinal plants of family Apiaceae. Many other

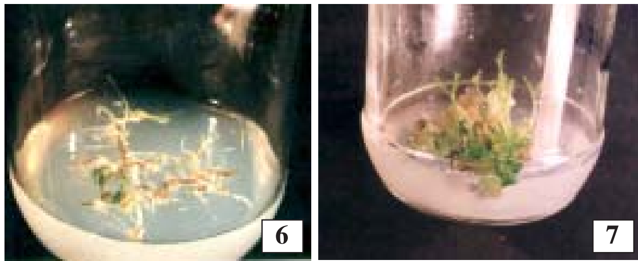


**Fig 6:** 9 week old root and callus from stem explant of *D. carota* on MS + NAA (1 mg/l).

**Fig 7:** 12 week old callus proliferating multiple shoots from stem explant of *D. carota* on MS + NAA (1mg/l) + BAP (2 mg/l).

economically important medicinal plants of family Apiaceae are propagated *in vitro* by using different explant and different propagation methods (Kim *et al*., 1996, Joshi *et al*., 2003). Pant *et al*. (1996), reported the formation of multiple shoot in *Cnidium officinale* by shoot tip culture. In *D. carota* though various parts were found to be totipotent to regenerate, the nodal explant and stem explant has been considered to be the appropriate explant for inducing the multiple shoots in the present investigation. Direct as well as indirect plant regeneration were obtained from the nodal and stem explants.

The root explant produced callus in most of the hormone concentration. At lower concentration of NAA and BAP, direct regeneration of callus was observed while at the higher concentration, rhizozenesis was formed followed by callus induction. The leaf segment of *D. carota* produced short roots on MS medium with NAA at both concentrations of 1 mg/l and 2 mg/l.. The addition of NAA in the medium has favored the production of roots in the leaf explant. Similar results were observed by Bekhi and Lesley (1976) and Kartha *et al.* (1976) working on *Lycopersicum esculantum*.

In the present study, in *D. carota* MS media alone was sufficient to regenerate the plantlet from the nodal explant. In MS medium supplemented with BAP (0.5 mg/l) multiple shoots were developed. In this condition highest number of multiple shoots were produced. When the concentration of BAP was increased to 1 and 2 mg/l, shoots were formed earlier. Hence, increase in concentration of BAP has positive effect on the earlier shoot formation, i.e. higher the concentration of BAP, less time taken for the formation of shoot. Sripichitt *et al*. (1987) observed that BAP was more effective than kinetin in inducing shoot formation on MS medium. The nodal explant of *D. carota* gave shoots and callus on MS medium supplemented with 0.5 mg/l NAA and further increase in the concentration of NAA, the nodal explant differentiated into the whole plant. Plant differentiated in short time period in 2 mg/l of NAA as compared to 1 mg/l of NAA. Thus it was observed that higher concentration of NAA has favored the plant differentiation more rapidly.

In the present investigation, in MS + NAA (0.5 mg/l) + BAP (0.5 mg/l), the nodal explant initiated the shoot formation followed by callus formation. Similar result was obtained when the concentration of BAP was increased to 1 and 2 mg/l. Joshi *et al*. (2003) also reported that MS medium supplemented with BAP (1 mg/l) + NAA (0.5 mg/l) was the ideal condition for the induction of micro shoots from the nodal segment of *Foeniculum vulgare*, a member of Umbelliferae.

Most of the work on tissue culture established that auxin like IAA, IBA, NAA induced root formation while cytokinin like BAP, Kinetin induce shoots and bud formation. Similarly in the present study, use of BAP alone and combined with NAA showed initiation of multiple shoots from the nodal explant of *D. carota*.

## CONCLUSION

By this experiment, it can be concluded that nodal and stem explants are the appropriate explants for the formation of multiple shoots in *D. carota*. MS medium supplemented with BAP (0.5 mg/l) and BAP (2 mg/l) + NAA (1 mg/l) were the ideal condition for the formation of multiple shoots from both the nodal and stem explants.

## ACKNOWLEDGEMENT

The authors are grateful to Central Department of Botany, Tribhuvan University, Kathmandu for providing laboratory facilities to conduct this research work. We highly acknowledge Prof. Dr. S. D. Joshi for her valuable suggestions.

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