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SHOOTS REGENERATION IN CALLUS TISSUE OF CORIANDER PLANT (*CORIANDRUM SATIVUM L.*) *IN VITRO*

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Abstract

Coriandrum sativum is a medicinal plant used to treat numerous such as diuretic and diaphoretic, treatment of disorders of respiratory and digestive system. The study examined in begin the coriander seeds cultivated were purified to (MS) medium devoid of plant growth regulators on seed germination until middle in length 2 cm then transfer plantlets to the new medium for developing induction callus so tissues using the new (MS) medium equipped with an organizer. The study effect of supplementing of auxin and cytokinin, benzyladinine (BA) 1.0 mg/l and 2, 4-dichlorophenoxyacetic acid (2,4-D) 0.5 mg/l in combination to Murashige and Skoog (MS) medium *in vitro.* After of appears callus, callus culture was induced regeneration of green branches of tissue callus on MS medium supplemented of (BA) and (2,4-D) in combination. Maximum callus induction was about (100%) culture.

***Keywords*:** *Coriandrum sativum*, Callus, MS, Auxin and Cytokinin.

Introduction

Medicinal plants play a major role in human health and being an important source of many medicines and pharmaceuticals, and lie attention medical these plants in their ability to produce many of the chemical compounds as well as characteristics pharmaceutical (Taylor *et al*., 1988).Coriander (*Coriandrum sativum* L.) is one of the medicinal plants belonging to the family Apiaceae (BenFredj *et al*., 2013). Coriander has carminative, stimulant, diuretic and diaphoretic activity so it is used in disorders of respiratory, digestive and urinary systems in the traditional medicine (Oudah and Ali, 2010). It also has been indicated for a number of medical problems such as convulsion, dyspeptic complaints. Coriander has been reported to possess several pharmacological activities like anti-diabetic (Eidi *et al.,* 2012), anti-oxidant (Darughe *et al*., 2012), anti-mutagenic (Cortes-Eslava *et al*., 2004), anti-lipidemic (Sunil *et al*., 2012), anti-spasmodic (Alison and Peter, 1999). The Umbelliferae (Apiaceae) family and is used as aromatic and medicinal plant as anti-spasmodic, appetite stimulant, stomachic, diuretic, anti-infilamentary and anti-diarrheic agent.

Classification of Coriander (*Coriandrum sativum L.*)

The taxonomical position was as the following:-

Kingdom : Plantae – Plants
Division : Angiospermae – Flowering plants.
Class : Magnoliopsida – Dicotyledons.
Order : Apiales- Umbellales.
Family : Apiacae
Genus : *Coriandrum sativum* L.

Fig. 1 : Coriander plant

The classification depending on (Priyadarshi and Borse, 2014; Rajeshwari and Andulla, 2011).

Regeneration methods of plants in culture:-

1- Organogenesis

Organogenesis is the creation of organs depends on the ratio of auxin and cytokinin and the ability of the tissue to respond to plant growth regulator during culture, organogenesis in vitro can be two types (Stewart, 2008):-

a- Indirect organogenesis

b- Direct organogenesis

2- Somatic organogenesis

Somatic embryogenesis is a process whereby somatic cells differentiate into somatic embryo. Somatic embryos look zygot embryos morphologically (George *et al*., 2008) ,which are bipolar structures arise from individual cells (Sharma and Agrawal, 2012)

Somatic embryogenesis two types:-

a- Direct somatic embryogenesis

b- Indirect somatic embryogenesis

Factors effecting of plant tissue culture:-

a- Choice explant

b- The culture medium used
c- Sterile condition
d- Growth regulators
e- Vitamins
f- Medium pH
g- Carbon source

Materials and Methods

Seeds viability and sterilization

Take amount of seeds were placed in container and washed with distilled water to eliminate of dust and dirt, after then sterilized by immersing in the sodium hypochlorite (clorex) 6% and shake about 4 minutes, and then washed of distilled water about 3-4 times, add 70% ethyl alcohol and shake quickly for 10second and washed with sterile distilled water for one minute at three- four times to eliminated of ethyl alcohol, then the seeds were placed in a petri dish containing filter paper in order to absorb wedged water after 4-5 days show the seeds growth in a petri dish.

Fig. 2 : Seeds germination

Seeds Culture

After preparation of MS medium without plant growth regulators, sterilize seeds *in vitro* were culturing one –two sterilize seeds in each tube containing 10-15 ml of sterile medium and incubated under light conditions 16 hours day and 8 hours night at temperature of 25±2°C and intensity illumination of 1000 lux. through fourteen days to twenty one days show growth of seedlings were used as a source for callus induction.

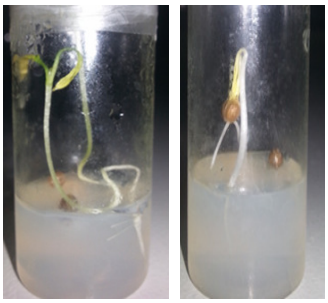

Fig. 3 : Plantlets growth in MS

Plantlet transfer and callus induction

Plant lets were cultured using modified methods, plantlet (2cm segments length) were surface sterilized with 70% (v/v) ethanol alcohol for 1 min followed by 6% (v/v) Clorox for 7 min then plantlet washed three times with sterilized distilled water. The plantlet were transfer on MS medium containing 30% sucrose,7g/l agar and supplemented with concentrations of BA 1mg/l and 2,4-D 0.5mg/l and pH=5.7-5at 25 ± 1°C under dark conditions. Plant growth regulator-free medium was used as the control treatment. After forty five days, callus induction frequency (100%).

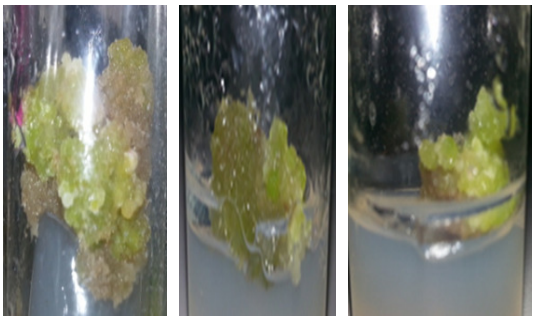

Fig. 4 : Callus induction of MS media

Sub-Culture of Callus

After fifteen days of plantlet transfer in MS medium, callus started to emerge and after forty five days the callus growth was completed. Where fresh weight of callus induced from the seeds was treated for 0.5 $mg.l^{-1}$ of 2,4-D and 1 $mg.l^{-1}$ of BA used in experiment. Callus was harvested from glass tubes and measured fresh weight using an electric sensitive balance after removal of medium residue suspended on callus by washing with sterilize distilled water. Taken two hundred mg of callus tissue, the developing fresh callus was weighted and sub-cultured on MS media at same concentration of plant growth regulators. For 25 repeats were cultured. Culturing process was made at sterilized conditions then callus tissue was development after forty five days of culturing show emerge of vegetative branches from callus cultured.

Fig. 5 : Regeneration of callus

Results and Discussion

In this experiment it included sterilization of coriander seeds (*Coriandrum sativum L.*) and cultured on Murashige and Skoog medium in half strength for seeds germination. After 21day, concentrations of growth regulator auxin 2,4-D (0.5mg/l) with cytokinin BA (1.0mg/l) were used to induce callus from coriander sterile seedlings. Callus was subcultured on MS medium supplemented with the combination of 2,4-D(0.5mg/l) and BA (1.0mg/l). After forty five days of culture, multiple shoots were regenerated from seeds treated with concentrations (1.0mg/l) BA and (0.5mg/l) 2.4-D in combination. Significantly higher mean of shoot length was recorded in seeds treated with 2.4-D and BA about 6.0 cm in length.

The results showed that the MS solid medium is the best media for seed germination and considered adding 2,4-D and BA are important for callus induction and regeneration from plantlets coriander with plant regulations, characterized was green color callus adopted the vegetable part type and concentration of growth regulators used, the commingling between growth regulators helps to regeneration of shoots from callus. Callus were sub cultured in the same medium to get enough amount for following experiments in growth room at 25±1 ºC. MS medium without plant growth regulator was the optimum culture condition for *in vitro* regeneration of *Corindrum sativum* L. from seeds. The optimum plant growth regulator for callus induction and further callus proliferation was 2,4-D and BA. The established regeneration and callus induction protocol provides a baseline for further studies on the breeding of the multipurpose plant as well as utilization of cell cultures for potential production of bioactive secondary metabolites.

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