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Effect of growth regulators on *In vitro* organogenesis in cauliflower (*Brassica oleracea* L. var. botrytis)

Nasmin Banu CV[1], P Indulekha[2]
[1, 2] Department of Botany and Research Centre, The Zamorin's Guruvayurappan College, Kozhikode, Kerala, India

Abstract
Cauliflower (*Brassica oleracea* l. var. botrytis) is a member of family Cruciferae (Apiaceae), and is cultivated all over North and South India. The whole inflorescence forms a large head of flowers on thick hypertrophied branches which are eaten as vegetable. Cauliflower contains Sulforaphane, which was shown to kill cancer stem cells. It also relieves high blood pressure. It contains antioxidants like vitamin C, and beta carotene. Conventional propagation takes a long period for multiplication due to poor rate of fruit set; poor germination and heterozygosis through seeds. *In vitro* culture includes culturing of cells, tissues, organs under aseptic laboratory conditions in culture media. Plant parts known as explants are cultured in nutrient medium. Plant tissue culture can overcome these problems and establish plants successfully through a standardized protocol. MS (Murashige and Skoog, 1962) basal medium was used for callus culture and organogenesis. The Auxin and Cytokinin used for leaf explants were NAA (Naphthalene Acetic Acid) 3 mg/L and BAP (Benzyl Amino Purine) 0.5 mg/L. MS medium +NAA 3 mg/L and BAP 0.5 mg/L was proved to be a favorable medium for callus induction, proliferation and shoot organogenesis.

Keywords: *Brassica oleracea* L. var.botrytis, plant tissue culture, callus induction, shoot organogenesis

1. Introduction

In vitro culture includes culturing of cells, tissues, organs under aseptic laboratory conditions in artificial culture media. Cell culture provides valuable informations on morphogenesis and plant development. *In vitro* culture includes culturing of cells, tissues, organs under aseptic laboratory conditions in culture media. Plant parts known as explants are cultured in nutrient medium.

Cauliflower is a member of family Cruciferae (Apiaceae), and is cultivated all over North and South India. It was introduced to India from England by British. In this plant a short erect stem is produced with an undeveloped inflorescence. The whole inflorescence forms a large head of abortive flowers on thick hypertrophied branches. 'Curd' is a word used to describe the head of a cauliflower. The condensed inflorescence is eaten as vegetable.

Cauliflower contains Sulforaphane, which was shown to kill cancer stem cells [8]. It also relieves high blood pressure. It was observed that protective potential of *Brassica oleracea* can be used against oxidative damages in cells *in vitro* and *in vivo* [1]. Cauliflower is rich in vitamins and minerals. As a good source of choline, it has a significant role in brain development. It contains antioxidants like vitamin C, and beta carotene. Cauliflower helps your body's ability to detoxify in multiple ways. It contains antioxidants that support detoxification along with sulfur-containing nutrients important for detoxification activities [7]. The glucosinolates in cauliflower also activate detoxification enzymes [3].

Cauliflower is relatively difficult to grow than cabbage. Conventional propagation takes a long period for multiplication due to poor rate of fruit set, poor germination and heterozygosis through seeds [6]. Plant tissue culture can overcome these problems and establish plants successfully through a standardized protocol.

2. Materials and Methods

The explants of *Brassica oleracea* L. var. botrytis were collected from actively growing healthy plants from the GREEN VEG organic farm, Kozhikode, Kerala. The tender leaflets (especially 3rd and 4th from the apex) from the active flushes of *Brassica oleracea* L. botrytis were used as explants. The explants were initially washed with running tap water for 1-2 hours and treated with Teepol prior to its treatment with disinfectant solution. These were then surface sterilized with mercuric chloride (0.1%w/v) for 5-10 minutes inside in the laminar air flow chamber and later thoroughly washed 4-5 times in sterile distilled water. The leaf explants were prepared by cutting the leaves into 1cm^2 pieces including the midrib.

2.1 Culture Medium

MS basal medium [5] was used for callus culture and organogenesis. The Auxin and Cytokinin used for leaf explants were NAA and BAP. Double distilled water was used for the preparation of stock solution, medium and for rinsing glassware. The pH of the prepared media was adjusted to 5.8 ± 0.1. The nutrient media was sterilized by autoclaving at 121°C and 16 psi for 15-20 minutes.

3. Results and discussion

In *Brassica oleraceae* var. botrytis the explants used were tender leaves for callus induction and indirect organogenesis. In two different media combinations MS + NAA 3mg/l + BAP 0.5m g/l and MS + NAA 0.5mg/l + BAP 3mg/l used, the explant showed different responses.

In MS + NAA 3mg/l + BAP 0.5 mg/l the rate of callus proliferation was higher. From an initial fresh weight of 0.40g the fresh weight increased to 2.16 g within 20 DAI. The growth index was calculated as 440 %, and was

increased to 3.518 within 30 DAI, and growth index was calculated as 779.5%.

3.1 Indirect shoot organogenesis

In 26% of the callus cultures, in MS + NAA 3mg /l +BAP 0.5 mg/l indirect shoot organogenesis was obtained within 35 DAI. The average number of shoots originated was 3.8. Among the cultures 6 shoots were obtained in 2 tubes each. Shoot organogenesis was initiated within 30-35 DAI, as spherical green colored out growths and later developed into plantlets with green leaves.

Table 1: Rate of callus proliferation in different media combinations in leaf explants of *B. oleraceae* var. botrytis

Culture Media	Initial Fresh Weight(g)	DAI (Days After Inoculation)	Final Fresh Weight (g) [1]	Growth Index%[2]	Average No. of Shoots
NAA(3mg/l) BAP (0.5mg/l)	0.40g	20	2.16 g	440	3.8
		30	3.518 g	779.5	
NAA(0.5mg/l) BAP (3mg/l)	0.40g	20	1.488g	272	0.0
		30	2.300g	475	

1. Mean of 25 replicates

2.

$$\text{Growth index \%} = \frac{\text{Final fresh weight} - \text{Initial fresh weight}}{\text{Initial fresh weight}} \times 100$$

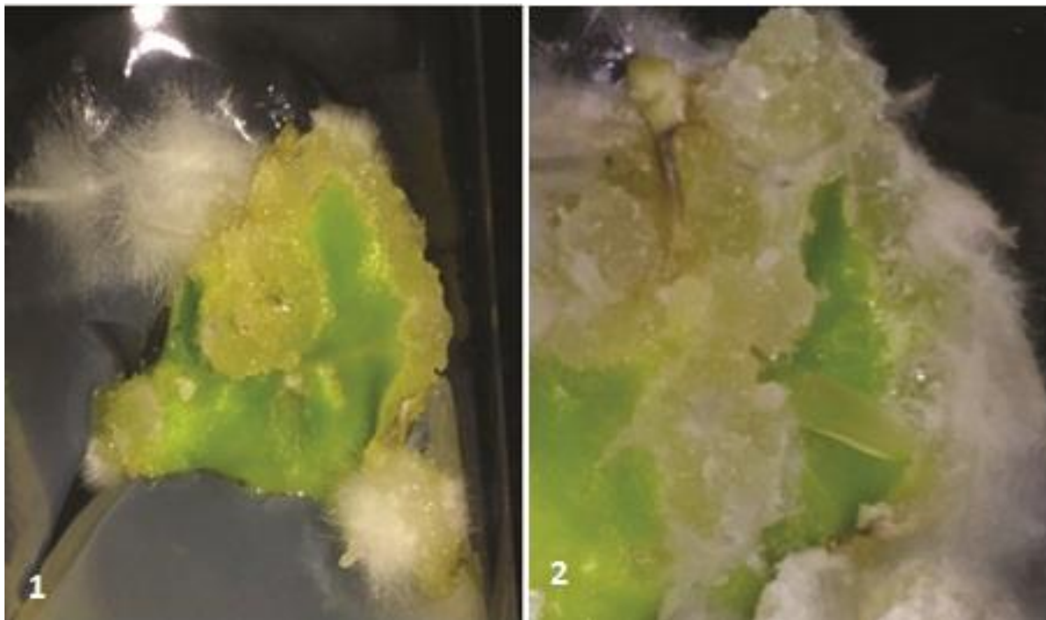

Fig 1&2: Callogenesis in MS + NAA 0.5 mg/l BAP 3.0 mg/l 20DAI and 30 DAI

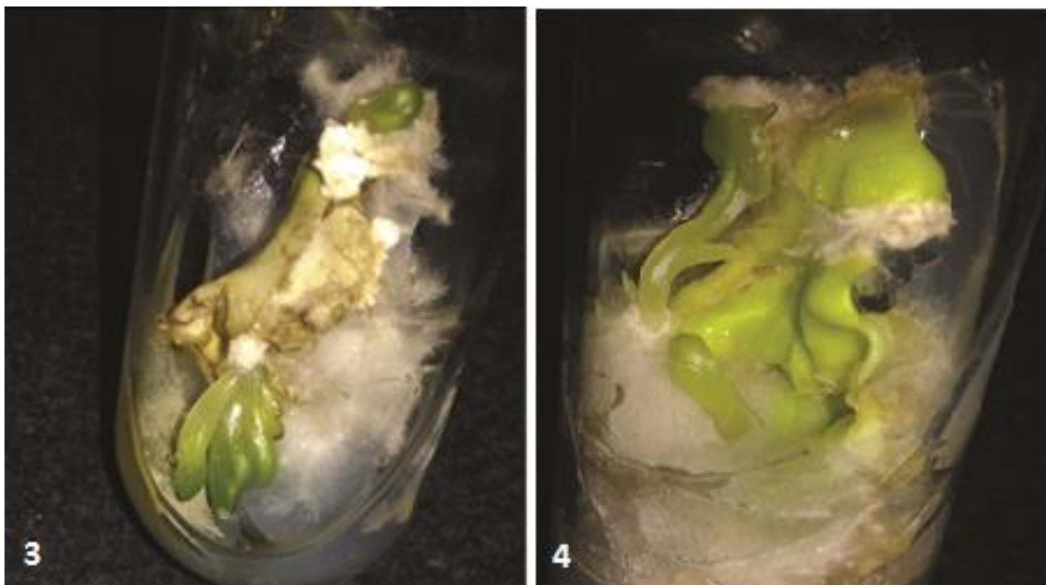

Fig 3&4: Indirect shoot organogenesis in MS + NAA 3.0 mg/l BAP 0.5 mg/l

In MS + NAA 0.5 mg/l BAP 3 mg/l the fresh weight was increased from initial fresh weight of 0.40g to 1.488 g within 20 DAI, the growth index was calculated as 272% and increased to 2.3g within 30 DAI and the growth index was calculated into 475%. In medium NAA 0.5 mg /l BAP 3 mg/l there was no organogenesis, but callus showed proliferation only.

In *Brassica oleraceae* var. botrytis, NAA 3 mg/l and BAP 0.5 mg/l proved to be an ideal medium for callus proliferation and the subsequent indirect shoot organogenesis, compared to NAA 0.5mg/l and BAP 3mg/l.

High frequency organogenesis with maximum response from petiole explant of *Brassica oleracea* was reported with NAA and BAP [4]. In *Brassica oleracea* var *capitata* regeneration was observed in MS medium with NAA and BAP [2]. The results obtained in the present study was in agreement to these earlier studies [4, 2].

4. Conclusions

1. In *Brassica oleraceae* var. botrytis tender leaf explant was found to be suitable for callus induction, proliferation and shoot organogenesis.
2. MS +NAA 3mg/L and BAP 0.5mg/L was proved to be a favorable medium for callus induction, proliferation and shoot organogenesis. In 26% of the callus cultures, regeneration in the form of shoot organogenesis was obtained.
3. Shoot organogenesis with root induction can be effectively used for the establishment of large number of seedlings. Shoot organogenesis can be used for the production of disease free propagules irrespective of seasonal fluctuations, throughout the year.

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