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IN VITRO RESPONSE OF *BRASSICA OLERACEA* L VAR BOTRYTIS (CAULIFLOWER)

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ABSTRACT

The use of leaf explants of cauliflower to regenerate disease free ,purebred lines were examined. Best results were obtained with tender leaf explants on MS medium with 6-benzylaminopurine (cytokinin)(0.5mg/l) and α-napthaleneacetic acid(3mg/l). An efficient method of artificial mass propagation was optimized for very nutritious vegetable,the cauliflower. This technique can be used to raise the disease free stock of cauliflower and for genetic improvement of in-vitro bulk of varieties on commercial scale in a very brief time span.

Keywords: cauliflower, in vitro culture, in vitro response, tissue culture, propagation, in vitro culture.

I. INTRODUCTION

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. Explants may be roots, hypocotyls, cotyledons, leaves, shoot apices, nodal segments, anther, embryos, endosperm, ovary or ovules.

An important aspect of all plant biotechnology processes is culture of plant cells or tissues and organs in artificial medium. One of the problems in conventional plant breeding is the range of organisms among which genes can be transferred, because of species barriers. Tissue culture has broken many of these barriers. Cell culture provides valuable information on morphogenesis and plant development. Studies on molecular, physiological and biochemical aspects of cells in culture have contributed to in depth understanding of cytodifferentiation, organogenesis and somatic embryogenesis. Micro propagation is another important morphological application of plant tissue culture, where small amounts of tissues can be used to raise thousand of plants. Culture medium contains proper quantity of micro and macro nutrients, amino acids, vitamins and hormones, like auxins and cytokinins. Commonly used medium is MS medium (Murashige and Skoog, 1962). Other media which are commonly in use are Gamborg's B5 Medium, White's Medium and SH Medium. A culture medium requires Sucrose or Glucose as source of carbon. Cauliflower is a member of family Cruciferae (Brassicaceae). It was originated from Asia minor. In 16th century it was introduced to France and England. It was introduced to India in 1822 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 edible head of a cauliflower. White head looks like a miniature tree on the cross section. It consists of densely packed curds. It is cultivated all over Northern India.

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II. OBJECTIVES

1. For the production of disease free propagules irrespective of pathogen infection and season.

2 .Tissue culture is used as a supplementary method to the conventional propagation methods in the nurseries on commercial scale in a very brief time span.

3.To regenerate purebred lines for crossing .

III. MATERIALS AND METHODS

All the inoculation steps were carried out in a sterile laminar air flow chamber fitted with a cool white fluorescent lamp and a UV lamp. The UV lamp was switched on for 30 minutes prior to the aseptic manipulations. The tender leaf segments were placed in such a way that their abaxial surfaces were in contact with the medium.

Table 1: Concentration of hormone used for *Brassica oleracea* L. *botrytis* **explants.**

PLANT	EXPLANT	BASAL MEDIUM	AUXIN	Concentration (mg/l)	CYTOKININ	Concentration(mg/l)
Brassica oleracea L. var.*botrytis*	Leaf	MS	NAA	3	BAP	0.5
			NAA	0.5	BAP	3

The stock solution of 2,4-D, NAA and BAP were prepared at concentration of 1mg/ml each. The required quantity of growth hormone was first dissolved in minimum quantity of alkaline double distilled water and made up to the final volume by adding double distilled water. For the preparation of nutrient medium, the required volumes of each stock solutions, $CaCl_2$ (10x), Iron source (100x), vitamin and others (100x) was taken using measuring cylinders and pipettes.

The complete dissolution of each component was ensured before the addition of next. It was followed by the addition of the carbon source (sucrose 3% w/v) and growth regulators. After dissolving all the ingredients with the help of a magnetic stirrer, (Remi equipments) the medium was made up to the final volume by adding double distilled water.All the cultures were incubated under controlled conditions of temperature, light and humidity in the culture room provide with culture racks. For callusing, the tender leaf segments were maintained in complete darkness at a temperature of 25^{o}C +- 1^{o}C and relative humidity of 70%. The cultures were observed regularly at an interval of 5 days. The percentage of responding explants nature of response including tissue enlargement, callusing, change in color and texture of calli was noted. Photographs were taken. The fresh weights were recorded at an interval of 5 days and growth index (GI) was calculated using the following formula,

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

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IV. RESULTS

a) HISTOLOGICAL STUDY

Internal structure of callus was analyzed for histological evidences. Squashes as well as hand sections of callus were prepared and stained with safranine and observed under microscope. Photographs were taken using a Motic image plus digital camera, connected to a high resolution microscope and PC at 100x.

b) CALLUS INDUCTION

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 for callus induction and differentiation, the explant showed different responses .

Table 2: Rate of callus induction in *Brassica oleraceae* var.*botrytis* leaf explants in two media concentration .

S.No	Explant	Media Combination	Time Period	Frequency Of Callus Induction	Callus characteristics
1	Tender leaf	MS+NAA (3mg/l)BAP (0.5mg/l)	15 DAI	45%	Faster induction and proliferation, off white in color
2	Tender leaf	MS+BAP(3mg/l)NAA (0.5mg/l)	25 DAI	35%	Slower induction and proliferation off white in color

The rate of contamination was manageable in both the media. In MS+NAA 3mg/L and BAP 0.5 mg/L the leaf explants showed faster callus induction, within 15 DAI (Days After Inoculation). In MS+NAA 0.5 mg/L + BAP 3 mg/L the callus induction was slower, after 25 DAI .

c) CALLUS PROLIFERATION

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Table 3: Rate of callus proliferation in different media combinations in leaf explants of *B. oleraceae* var.*botrytis* L.

Media	Fresh Weight Initial	DAI	Final Fresh Weight*	Growth Index%
NAA(3mg/l) BAP (0.5mg/l)	0.40g	25	2.16g ± 0.5	440
		35	3.518g ± 0.6	779.5
NAA(0.5mg/l) BAP (3mg/l)	0.40g	25	1.488g ± 0.5	272
		35	3.200g ± 0.5	700

* mean of 25 replicates ± SD

** **Growth index % = final fresh weight - initial fresh weight x 100**

Initial Fresh Weight

In MS + NAA 3mg/l + BAP 0.5 mg/l rate of callus proliferation was higher. From an initial fresh weight of 0.40g the fresh weight increased to 2.16 g within 25 DAI. The growth index was calculated as 440 %, and was increased to 3.518 within 35 DAI, and growth index was calculated as 779.5%.

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 25 DAI, the growth index was calculated as 272% and increased to 3.2g within 35DAI and the growth index was calculated into 700% .

d) MORPHOLOGY OF CALLUS

In both media combinations the callus was noticed to arise from the margins as well as midrib of leaf explant .The callus proliferated into all the two types hard and friable . Among these the shoot organogenesis was obtained in friable callus alone. This callus was loosely attached to the mother explant .

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e) SHOOT ORGANOGENESIS

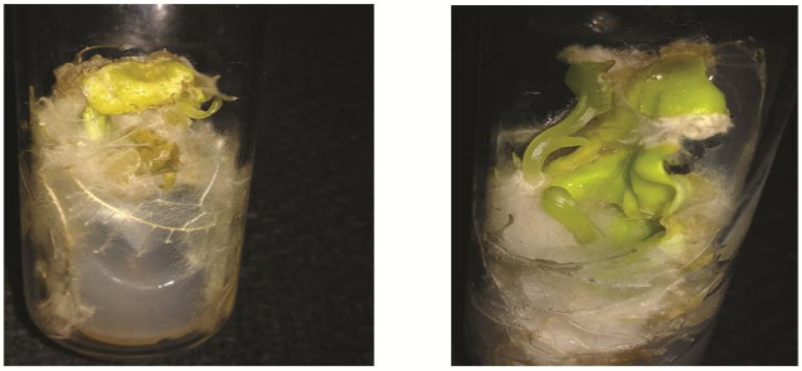

Fig 1;Shoot organogenesis at various stages.

In 15% of the callus cultures, indirect shoot organogenesis was obtained in the same medium. The mean number of shoots originated from medium MS + NAA 3mg /l +BAP 0.5 mg/l was 3.8. Among the cultures 6 shoots were obtained in 2 tubes each. In medium NAA 0.5 mg /l BAP 3 mg/l there was no organogenesis, but callus showed proliferation only. Shoot organogenesis was initiated within 30-35 DAI, as spherical green colored out growths and later developed into meristemoids. The meristemoids then developed into plantlets with green leaves.

In almost 70% of the cultures in which callus proliferation was obtained rhizogenesis was observed.

Table 4: Rate of shoot organogenesis in ***Brassica oleracea* var *botrytis* leaf explant**

Sl no	MEDIUM	Mean Number of shoots *
1	MS + NAA3mg/l BAP 0.5mg/l	3.8*
2	MS + NAA 0.5mg/l BAP 3mg/l	0%

* Mean of 20 replicates.

f) HISTOLOGY OF CALLUS

Histological section of the friable callus in which shoot organogenesis was induced in MS + NAA 3mg/l + BAP0 0.5mg/l showed meristemoids as compact masses . In *Brassica oleraceae* var .*botrytis* for callus induction, NAA 3 mg/l and BAP 0.5 mg/l proved to be a better medium. In the case of callus proliferation and the subsequent indirect shoot organogenesis also, MS + NAA 3 mg/l and BAP 0 .5 mg/l was more desirable, compared to NAA 0.5mg/l and BAP 3mg/l. Results obtained in the present study was similar to the studies of Gerszberg *et al*. (2015). They have reported regeneration in *Brassica oleracea* with NAA and BAP.

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V. CONCLUSION

Brassica oleraceae var.botrytis is economically valuable as a nutritious vegetable which can be propagated by adopting plant tissue culture method. Tissue culture is used as a supplementary method to the conventional propagation methods in the nurseries. *Brassica oleraceae var. botrytis* tender leaf explants was found to be a suitable explant for developing a standard protocol for callus induction , proliferation and shoot organogenesis .MS +NAA 3mg/L and BAP 0.5mg/L was proved to be a favorable medium for callus induction, proliferation and shoot organogenesis.Shoot organogenesis with root induction can be effectively used for the establishment of large number of seedlings .This method can be exploited for the production of disease free propagules irrespective of pathogen infection and season.

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