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Root, shoot and callus cell proliferation from broccoli root tip, shoot tip and leaf cutting *in vitro*

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Abstract
Background and objective: Cell or tissue culture as micro-propagation from stem, leaves, root, crown, sucker or embryo etc. has been successfully done in plant tissue culture Biotechnology. The study was conducted to investigate the root, callus, shoot and leaf proliferation from the root, shoot tip and leaf cutting.
Materials and Methods: The MS media with IBA and BAP were used as rooting media. The media with hormones prepared for five replicates of each hormone concentration. BAP and IBA concentrations were 0, 0.25, 0.5, 1.0, 2.0, 3.0 mg/l.
Results: The results showed that root, shoot, leaf and callus cell and tissue proliferation were regenerated successfully. The highest number of root proliferation was found (1.8) in the concentration of IBA 2.0 + BAP 2.0 mg/l combination cultured from root tip. However, the maximum root proliferation was found in the concentration of IBA 2.0 + 1.0 BAP combination cultured from leaf cutting and shoot tip. Positively callus formation was found better in the concentration of BAP 1-3.0 and IBA 1-3.0mg/l combination than other combination of concentrations in the case of root, shoot tip and leaf cutting. Moreover, the leaf proliferation was found better in the concentration of IBA 2-3.0 + 1-3.0mg/l BAP combination cultured from root tip, leaf cutting and shoot tip than other combination of concentrations. The highest callus weight was found in the cultured from shoot and leaf cutting than root cutting in the concentration of IBA 2.0 + 1.0 mg/l BAP combination.
Conclusion: The results conclude that it was better to use the combination of the IBA and BAP in the concentration of 1.0-3.0 mg/l to regenerate root, shoot, leaf and callus cell proliferation of broccoli from root tip, shoot tip and leaf cutting.

Keywords: root, shoot, callus cell, broccoli

Introduction

In vitro vegetable culture is an superlative branch of horticultural biotechnology as the broadness of applied plant biology. In the modern technology age, tissue culture generally refers to the growth of cells from a tissue from a multicellular organism *in vitro*. These cells may be cells isolated from a donor organism, primary cells or an cell line. The cells are suspended in a culture medium, which contains essential nutrients and energy sources [1]. Cell or tissue culture as micro-propagation from stem, leaves, root, crown, sucker or embryo etc has been successfully done in plant tissue culture Biotechnology. Plant tissue culture relies on the fact that many plant cells have the ability to regenerate a whole plant called as totipotency [2]. A single cells, plant cells without cell walls (protoplasts), pieces of leaves, or (less commonly) roots can often be used to generate a new plant on culture media given the required nutrients and plant hormones. Modern plant tissue culture is performed under aseptic conditions under filtered air[3]. Millions of ornamental, vegetable or fruit plant like pineapple propagule can be produced by tissue culture from root, leaves, crown or stem per year [3]. The rate of multiplication and total number of plantlets production were reported and recommended by using plant growth regulators. A total number of plantlets production ranging from 5000 [4]; 40000 [5]; 100000 [6] from single explant per year. Propagation of plant can be gained *in vitro* treated with BAP alone [7], mixture of hormones like BAP and naphthalene acetic acid (NAA) [8], indole butyric acid (IBA) [9], indole acetic acid (IAA) [10] and 2,4-dichlorophenoxy acetic acid (2,4-D) [5], combination of BAP and two auxins as NAA and IAA [11], IAA and IBA and IBA. Application of BAP alone was cost effective and could be useful over combination of two and three hormones [12]. Moreover, the optimum concentration of BAP was not yet recommended extensively. BAP at the concentration of 1.0 [7], 2.0 [13], 2.5 [14], 3.0 [8] and 4.0 mg/l [15] were recommended for multiplication of plantlet. It was reported [16] that the using wider concentration range in caster bean increased castor proliferation rate five times higher. They also reported that wider concentration range and mixture of hormones were not recommended now-a-days by Environmental biologists although it showed better shoot formation but, due to the pollution and not cost effective, it may be avoided. This study was done using the broccoli root, shoot tip and leaf cutting. There are not available literatures found on the present research. Therefore the following objectives were undertaken

1. To regenerate broccoli plants from explants of broccoli root, shoot tip and leaf cutting
2. To find out the effect of the different concentration of IBA and BAP on the roots, shoot leaf and callus cell formation from broccoli root, shoot tip and leaf cutting.

Materials and Methods

Preparation of Murashige and Skoog (MS) Basal Media

The MS basal media [17] were used as control and seed germination was prepared follow the standard procedures for MS powder (4.4g) form preparation. MS powder form was added in a beaker filled with 800 ml distilled water. Then followed up with 30 g of sucrose, adjusted the pH (5.8) and added 2.8 phyta gels. The media were made until 1000 ml volume.

Media in the Autoclave

MS basal media with auxin prepared by adjusted the pH to 5.8 by using 1 N HCL and 1 M NaOH. Then, the media was fractional in 30 ml into jam jars and autoclaved at 15 psi and 121˚C for 20 minutes. After that, the sterilized media were cooled and kept in culture room under dark condition. Preparation of media was done a week before use.

Seed Sterilization and Germination in the MS Media

Seeds of *broccoli* were obtained from the nursery. A total of 350 seeds were used to culture on MS [5] basal medium. About 70 jam jars were used to culture the seeds and about five seeds were germinated on every jam jars. The seeds were washed in 70% ethanol about 5 minute, and then rinsed in 15% chlorox about 15 minutes. The seeds were bringing into laminar flow and continued rinsed with sterile DH20 only for a few seconds. Then, the sterile seeds were germinated in MS basal media for 7 days. This process was carried out under aseptic condition in the laminar flow. The seeds were exposed to light cool white fluorescent tubes for a photoperiod of 16 hours in the incubation room at 25-28˚C.

MS basal media with IBA and BAP (2nd time media preparation)

The MS media with IBA and BAP were used as rooting media MS powder form was added in a beaker filled with 800 ml distilled water and 30 g of sucrose was added. Then, the hormones with specific concentration from stock solution were added by using micropipette. Adjusted the pH and 2.8 g phyta gel was added. The media were made until 1000 ml volume. The media with hormones prepared for five replicates of each hormone concentration. BAP and IBA concentrations were 0, 0.25, 0.5, 1.0, 2.0, 3.0 mg/l.

Root Culture on MS Supplemented with IBA and BAP

The roots were collected from seedling and root tips were cut and put into the media with ranging different hormone concentrations of IBA and BAP (0, 0.25, 0.5, 1.0, 2.0 and 3, 0 mg/l). There was Per treatment was consisted of five replications.

Shoot tip Culture on MS Supplemented with IBA and BAP

After one week of germination, seven days seedlings were selected as source of explants. The hypocotyls explants shoot tip were cut and transferred into media with different concentrations of IBA and BAP (0, 0.25, 0.5, 1.0, 2.0, 3.0 mg/l). Each treatment was consisted of five replications.

Leaf cutting slice Culture on MS Supplemented with IBA and BAP

After one week of germination, seven days seedlings were selected as source of explants. The hypocotyls explants (leaf slice) transferred into media without auxin (control) and media with varying levels of IBA and BAP (0, 0.25, 0.5, 1.0, 2.0, 3.0 mg/l). Each treatment was consisted of five replications.

Data collection

Callus cell proliferation from root tip, shoot tip and leaf cutting and weight were measured.

Statistical Analysis

Statistical analysis of the data was carried out by using analysis of variance (ANOVA) and differences among treatment means were compared by using Least Significance Difference (LSD) Test at 5% probability level.

Results

Root proliferation, Callus formation and leaf proliferation

There was higher formation of root observed from the leaf and shoot cutting in different concentration of IBA and BAP compared to the root tip cutting (Table 1). The highest number of root proliferation was found (1.8) in the concentration of IBA 2.0 + BAP 2.0 mg/l combination cultured from root tip. However, the maximum root proliferation was found in the concentration of IBA 2.0 + 1.0 BAP combination cultured from leaf cutting and shoot tip (Table 1). The callus formation was found higher in the concentration of BAP 1-3.0 and IBA 1-3.0mg/l combination than other combination of BAP 0.25-0.5 and IBA 0.25-0.5mg/l in the case of root, shoot tip and leaf cutting (Table 2). In addition to that the leaf proliferation was found higher in the concentration of IBA 2-3.0 + 1-3.0mg/l BAP combination cultured from root tip, leaf cutting and shoot tip than other combination of concentrations (Table 3). The highest callus weight was found in the cultured from shoot and leaf cutting than root cutting in the concentration of IBA 2.0 + 1.0 mg/l BAP combination (Table 4). Fig. 1 shows the image of the broccoli culture procedure.

Table 1: Effects of IBA and BAP on roots formation from broccoli root tips, shoot tips and leaf cutting

IBA	BAP	Observation of root formation from root tip cutting	No. of root formation from leaf cutting	No. of root formation from shoot tip cutting
0	0.25	-	0	0.0 ± 0.0
	0.5	-	0	0.0 ± 0.0
	1.0	-	0	0.0 ± 0.0
	2.0	-	0	0.0 ± 0.0
0.25	3.0	-	0	0
	0.25	-	0	0
	0.5	-	0	1.0 ± 0.41
	1.0	-	0	2.5 ± 0.29
	2.0	-	0	2.25 ± 0.25

0.5	3.0	-	0	1.75± 0.48
	0.25	-	0	2.0± 0.71
	0.5		1.0 ± 0.41	1.5± 0.29
	1.0	-	1.75± 0.25	1.75± 0.49
	2.0	-	2.0 ± 0.41	1.5± 0.29
	3.0	-	2.5 ± 0.29	1.75± 0.48
1.0	0.25		2.25 ± 0.25	1.5± 0.29
	0.5	0.5 ± 0.01	1.05 ± 0.29	2.0± 0.41
	1.0	0.53± 0.04	1.25 ± 0.25	2.25± 0.48
	2.0	1.2± 0.02	1.5 ± 0.65	3.25± 0.75
	3.0	1.5± 0.01	2.0± 0.71	2.25 ± 0.48
		1.4± 0.02	1.5± 0.29	2.25 ± 0.25
2.0	0.25	0.6 ± 0.02	2.25 ± 0.48	2.0 ± 0.41
	0.5	0.55± 0.03	2.5± 0.65	2.75 ± 0.49
	1.0	1.6± 0.01	2.75 ± 0.65	3.5 ± 0.65
	2.0	1.8± 0.02	1.5 ± 0.65	2.5 ± 0.29
	3.0	1.7± 0.03	2.25 ± 0.25	3.5 ± 0.65
3.0	0.25	0.5 ± 0.03	1.5 ± 0.65	2.0 ± 0.41
	0.5	0.52± 0.01	2.5 ± 0.29	2.5 ± 0.29
	1.0	1.6± 0.03	3.5 ± 0.65	2.75 ± 0.25
	2.0	1.5± 0.02	3.0 ± 0.41	2.5 ± 0.65
	3.0	1.5± 0.03	2.75 ± 0.25	2.25± 0.25

Mean ± SE of 5 replicates. + = organ (root) formation was indicated. - no-indication of organ formation.

Table 2: Effects of IBA and BAP on callus formation from broccoli root tip, shoot tip and leaf cutting.

IBA	BAP	Observation of callus from root tip	Observation of callus from shoot tip	Observation of callus from leaf cutting
0	0.25	-	-	-
	0.5	-	-	-
	1.0	-	-	-
	2.0	-	-	-
	3.0	-	-	
0.25	0.25	-	-	-
	0.5	-	-	-
	1.0	-	-	-
	2.0		-	-
	3.0	-	-	-
0.5	0.25	-	-	-
	0.5	-	-	-
	1.0	-	-	-
	2.0	-	-	-
	3.0	-	-	-
	0.25	-	-	-
	0.5	-	-	-
1.0	1.0	Callus formed	Callus initiated	Callus formed
	2.0	Green and whitish	Green and whitish	Green and whitish
	3.0	Compact and globular	Compact and globular	Compact and globular
	0.25	-	-	-
	0.5	-	-	-
	1.0	Callus formed	Callus initiated	Callus formed
2.0	2.0	Green and whitish callus	Green and whitish callus	Green and whitish
	3.0	Compact and globular	Compact and globular callus	Compact and globular
	0.25	-	-	-
	0.5	-	-	-
	1.0	Callus formed	Callus initiated	Callus formed
	2.0	Green and whitish	Green and whitish callus	Green and whitish
3.0	3.0	Compact and globular callus	Compact and globular	Compact and globular

Mean ± SE of 5 replicates. + = organ (leaf) formation was indicated. - no-indicat

Table 3: Effects of IBA and BAP on the leaf proliferation from broccoli root tip, shoot and leaf cutting.

IBA	BAP	Leaf proliferation from root tip	Leaf proliferation from shoot tip	Leaf proliferation From leaf cutting
0	0.25	-	-	-
	0.5	-	-	-

	1.0	-	-	-
	2.0	-	-	-
	3.0	-	-	-
0.25	0.25	-	-	-
	0.5	-	-	-
	1.0	-	-	-
	2.0	-	-	-
	3.0	-	-	-
0.5	0.25	-	-	-
	0.5	-	-	-
	1.0	-	-	-
	2.0	-	-	-
	3.0	-	-	-
1.0	0.25	-	-	-
	0.5	-	-	-
	1.0			-
	2.0	-	-	-
	3.0	-	-	-
2.0	0.25	-	-	
	0.5	-	-	
	1.0	+	+	+
	2.0	+	+	+
	3.0	+	+	+
3.0	0.25	-	-	-
	0.5	-	-	-
	1.0	+	+	+
	2.0	+	+	+
	3.0	+	+	+

Mean ± SE of 10 replicates. + = organ (leaf) formation was indicated. - no-indication of organ formation. *P <0.05 is significantly different for each treatments obtained from One Way ANOVA test.

a. Broccoli growth b. Shoot and leaf c. Rooting d**.** Callus formation, Shoot and leaf proliferation

Fig 1: Photograph shows broccoli growth, callus and leaf proliferation

Discussion

From the results, it can be described that the leaf and shoot cutting showed higher root proliferation, callus formation, leaf proliferation, callus weight, carbohydrate represented as inverted sugar and glucose, chlorophyll and nutrient content (K+, NO3-, Ca++ and Na+) than in different concentration of IBA 2.0 + 2.0 mg/l BAP combination compared to the root tip cutting. These concentrations might not be suitable for root proliferation and callus formation. It was reported [18] that growth and morphogenesis of cell culture or organ were affected by genotype, substrate, environment and tissues have been used. It was reported [19] that the genotype which had the high capability was important to be chosen to produce good regeneration in tissue culture.

It was reported [2] that the suitable part to be cultured depends to the species and explants reaction also depends to different condition of the mother plants such as grow condition and age of The explants. Besides that, soft woody plant or non woody plant was easy to culture compared to woody plants. Plant tissue culture needs several organic chemical such as nitrogen, magnesium sulphate, phosphorus, natrium and chloride ion [20]. Different combination and concentration of hormone affects the plants growth. It was reported [21] that, the different concentration of auxin and cytokinin are important to roots, meristem and shoots for explants from meristems tissue of tobacco, banana [22] and pineapple [2]. Callus formation was obtained if the concentration of auxin and cytokinin was same [18]. For Brassica olerace var italica callus also obtained from media supplemented with different concentration of auxin and cytokinin [23]. which showed similar to the present results. In this study, the antioxidant activities of leaf extracts of broccoli were evaluated. Several different methods have been developed to evaluate the antioxidant activity of biological samples [24].

Based on the results, the leaf extracts showed the highest free

Radical scavenging potential, sugars, total antioxidant, total phenol, chlorophyll, and nutrient content than other extracts in the concentration which are IBA and BAP (2 mg/l and 2 mg/l). This is due to the different parts of the plant produce different Compounds or different amount of compounds due to their differential gene expression. Therefore, this particularly affects the antioxidant potential of the different parts of a given plant [25]. De-coloration due to reaction of antioxidants in extracts with the stable free DPPH radical was measured by spectrophotometer. 2, 2-diphenyl-2-picrylhydarzyl (DPPH) assay evaluates the ability of antioxidant to scavenge free radicals. Other factors that can increase or decrease the antioxidant compounds include samples condition and polarity of the extraction solvents [26].

In addition to that, it is well known that red and dark green colored leafy vegetables are richer in nutrient content than lighter colored vegetables. The naturally occurring compounds adequate for food colouring pigments, such as the chlorophyll, anthocyanins, betalains (betacyanin and betaxanthin) and carotenoids, are involved in leaf coloration. All of these components have been established to have antioxidant activities [26]. Hence, this proved that green colour of leaves can affects or increase the antioxidant activity of leaf extracts in this study.

Conclusion

The current results conclude that the best combination of the IBA and BAP in the concentration of 1.0-3.0 mg/l to regenerate root, shoot, leaf and callus cell proliferation of broccoli from root tip, shoot tip and leaf cutting. In addition, the highest sugars, total antioxidant, total phenol, chlorophyll, DPPH and nutrient content was found in the concentration of IBA and BAP (2 mg/l and 2 mg/l) than IBA and BAP (1 mg/l and 2 mg/l) and IBA and BAP (3 mg/l and 2 mg/l).

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