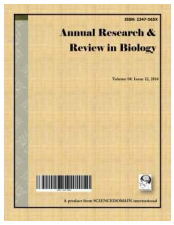

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Root Cell Proliferation from Broccoli Root Tips *in vitro* Culture Using Indole Acetic Acid (IAA), Indole Butyric Acid (IBA) and Benzylaminopurine (BAP)

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Authors' contributions

This work was carried out in collaboration between all authors. Author ABMSH designed the study, wrote the protocol and interpreted the data. Author NAI anchored the field study, gathered the initial data and performed preliminary data analysis. Authors MA and ABMSH managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

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ABSTRACT

Significance of the Study: Cell or tissue culture from root tips, shoot tips and leaf cuttings plays a significant role in micro-propagation in the plant production industry. Nowadays, it has been successfully done from many fruits, vegetables, ornamental and forest plants. Millions of explants can be produced by tissue or cell culture per year in any plant production industry.
Aim: The study was conducted to investigate the root, shoot and leaf formation from the root tip cultures using different IAA, IBA and BAP concentrations.
Methodology: IAA, IBA and BAP concentrations of 0.25, 0.50, 1.0, 1.5, 2.0, 2.5 mg/l combined with MS media were used in broccoli root tip culture.
Results: The results showed that root and callus formation were done successfully. But there was

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no shoot and leaf formation. The highest number (3.5) of callus proliferation was found in the concentration of IAA 1.5 and IBA 1.5 mg/l combination having BAP 1.0 mg/l. Moreover, root formation was found initially in the concentration of IAA 1-2.0 and IBA 1-2.0 mg/l combined with 1.5 mg/l BAP. In addition, later on it was found in the concentration of IAA 0.25-2.5 and IBA 0.25-2.5 mg/l combined with 1.5-2.5 mg/l BAP compared to other combination of concentrations.
Conclusion: Therefore present results showed that it was better to use the combination of the IAA, BAP and IBA concentration to produce root proliferation and callus formation of broccoli using root tip cultures.

Keywords: Cell and tissue culture; root tip; IAA; IBA; BAP.

1. INTRODUCTION

Micro-propagation is an important and rapid technique in producing or regenerating cell, callus, tissue, explants as well as plants via in vitro culture [1]. Plant tissue culture is a technique used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. Plant tissue culture is widely used to produce clones of a plant in a method known as micro-propagation. Different techniques in plant tissue culture may offer certain advantages over traditional methods of propagation [2].

Plant tissue culture relies on the fact that many plant cells have the ability to regenerate a whole plant known as totipotency. 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 and filtered air [3]. Propagation of plant can be gained in vitro treated with BAP alone [4] mixture of hormones like benzylaminopurine (BAP) and naphthalene acetic acid (NAA) [5], indole butyric acid (IBA) [6], indole acetic acid (IAA) [7] and 2,4-dichlorophenoxy acetic acid (2,4-D) [8] combination of BAP and two auxins as NAA and IAA [9], IAA and IBA [10] and IBA [11] Application of BAP alone was cost effective and could be more useful than a combination of two and three hormones. However, the optimum concentration of BAP has not yet been determined extensively, BAP at the concentration of 1.0 [5], 2.0 [11] 2.5 [12], 3.0 [7] and 4.0 mg/l [13] was recommended. This study was done using broccoli (*Brassica oleracea* var italic) root tip with different concentrations of IAA, IBA combined with BAP concentrations. There is no available literature on this topic. Therefore the following objectives were undertaken:

i. To regenerate broccoli root proliferation and callus from root tips via organogenesis.
ii. To investigate the effect of various concentration of auxin (IAA and IBA) and cytokinin (BAP) hormones on the roots and callus formation from broccoli root tips.

2. MATERIALS AND METHODS

2.1 Preparation of Murashige and Skoog (MS) Basal Media

The MS basal media [14] were used as control and seed germination was prepared following the standard procedures for MS powder form preparation (Table 1). MS powder form was added in a beaker filled with 800 ml distilled water to be followed by 30 g of sucrose and 2.8 phyta gels and adjustment the pH to 5.8 so that the final medium volume was 1000 ml.

Table 1. Standard procedures for MS media preparation

Component	Unit
MS powder form with vitamin	4.4 g
Sucrose	30 g
Phyta gel	2.8 g
pH	5.8

2.2 Media in the Autoclave

MS basal media with auxin was prepared by adjusting the pH to 5.8 by using 1 N HCl and 1 M NaOH. Then, the media was fractional in 30 ml and was added into jam jars (7 x 4.5 cm2) and autoclaved at 15 psi and 121℃ 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 to reduce water condensation in jam jars and the media was sterilized completely.

2.3 Seed Sterilization and Germination in the MS Media

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

2.4 MS Basal Media with IAA and IBA and BAP (2nd Time Media Preparation)

The MS media with IAA, 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. The pH was similarly adjusted and 2.8 g phyta gel was added. so that 1000 ml of medium was prepared. The media with hormones were prepared for five replicates of each hormone concentration. The BAP (synthetic cytokinin), IBA (synthetic auxin) and IAA (natural auxin) concentrations were 0.25, 0.5. 1.0, 1.5, 2.0, 2.5 mg/l.

2.5 Root Culture on MS Supplemented with IAA, IBA and BAP

The roots were collected from seedling and root tips were cut into pieces and put into the media with different hormone concentrations of IAA (0.25, 0.50, 1.0, 1.5, 2.0 and 2.5 mg/l), IBA and BAP (0.25, 0.50, 1.0, 1.5, 2.0 and 2.5 mg/l). There were five replicates per treatment.

2.6 Statistical Analysis

Standard deviation and then standard Error was made to compare the replicates.

3. RESULTS AND DISCUSSION

3.1 Root proliferation and Callus Formation

After two to three weeks of sub-culture, the roots showed the positive results by producing more roots on media with hormone but negative results for the formation of shoots. There was a low formation of roots observed from the root tips in different concentration (Table 2). But the formation of callus from root tips mostly gave the best response in media with combination of hormones. It was found that the root formation occurred at the concentration of 1.5 mg/l BAP combined with 1-2.5 mg/l IAA and IBA (Table 2). The initial callus formation, green and whitish callus and compact and globular callus formation were found at the concentration of IAA and IBA combined with BAP. The best callus formation (compact and globular callus) was found at the combination of concentrations of BAP 1.0 + IAA 2.0 + IBA 2.0 mg/l, BAP 1.5 + IAA 2.0 + IBA 2.0 mg/l, BAP 2.0 + IAA 2.0 + IBA 2.0 mg/ and BAP 2.5 + IAA 2.0 + IBA 2.0 mg/l (Table 2). Callus weight was higher (3.5) at the concentration of 1.0 BAP+1.5 IAA+1.5 IBA than other concentrations (Table 3). The second highest (2.5) was found at the concentration of 1.5 mg/l BAP+1.5 mg/l IAA+1.5 mg/l IBA. However, the lowest (1.3) was found at the concentration of 1.0 mg/l BAP+1.0 mg/l IAA+1.0 mg/l IBA (Table 3). Fig. 1 shows the root and callus formation from broccoli root tip.

3.2 Discussion

Our results demonstrated the optimization of the cell culture and root generation and callus proliferation. In the concentration of 0.25 and 0.5 mg/l of Bap, IAA and IBA, no root formation occurred. In addition in the concentration of 0, 0.25 and 0.5 mg/l of IAA IBA and BAP, there was no callus and root proliferation. However, overall shoot and leaves formation were not occurred. These might be due to the cell differentiation and division not happened to these concentrations. These concentrations might not be effective for cell differentiation and division for callus and root proliferation. These might be due to the cell differentiation and division not responsive to these concentrations. [9] It has been reported that 1.0 mg/l IAA and 1.0 mg/l combined with 0.25 mg/l gibberellic acid showed better root regeneration in potato than other concentration. [15] It has been reported that 1.0 mg/l IAA and 1.0 mg/l IBA combined with 0.3 mg/l auxin (NAA) showed better root regeneration in potato than other concentration. [12] It has been reported that 1.0 mg/l IAA and 1.0 mg/l IBA combined with 1.0 mg/l auxin (NAA) showed the best root, shoot and leaf regeneration in potato.

Different combinations and concentrations of hormone affects the plants growth [5]. It has

been reported that the different concentrations of auxin and cytokinin are important for the roots and shoots of explants from meristemic tissues of tobacco, banana [16] and pineapple [2].

Table 2. Effects of IAA IBA and BAP on roots and callus formation from broccoli root tips

BAP	IAA	IBA	Observation of root formation	Observation of callus
0	0	0	-	-
0.25	0.25	0.25	-	-
	0.5	0.5	-	-
	1.0	1.0	-	-
	1.5	1.5	-	-
	2.0	2.0	-	-
	2.5	2.5	-	-
0.5	0.25	0.25	-	-
	0.5	0.5	-	-
	1.0	1.0	-	-
	1.5	1.5	-	-
	2.0	2.0	-	-
	2.5	2.5	-	-
1.0	0.25	0.25	-	-
	0.5	0.5	-	-
	1.0	1.0	-	Callus formed
	1.5	1.5	-	Green and whitish callus
	2.0	2.0	-	Compact and globular callus
	2.5	2.5	-	-
1.5	0.25	0.25	-	-
	0.5	0.5	-	-
	1.0	1.0	+ (root)	Callus formed
	1.5	1.5	+ (root)	Green and whitish callus
	2.0	2.0	+ (root)	Compact and globular callus
	2.5	2.5	+ (root)	-
	0.25	0.25	+ (root)	-
2.0	0.5	0.5	+ (root)	-
	1.0	1.0	+ (root)	Callus formed
	1.5	1.5	+ (root)	Green and whitish callus
	2.0	2.0	+ (root)	Compact and globular callus
	2.5	2.5	+ (root)	-
	0.25	0.25	+ (root)	-
	0.5	0.5	+ (root)	-
2.5	1.0	1.0	+ (root)	Callus formed
	1.5	1.5	+ (root)	Green and whitish callus
	2.0	2.0	+ (root)	Compact and globular callus
	2.5	2.5	+ (root)	-

Mean ± SE of 10 replicates. + = organ (root) formation was indicated. - no-indication of organ formation.

a. Germinated plants from seeds b. Rooting c. Callus formation

Fig. 1. Photo shows the root and callus formation from broccoli root tip

Table 2 showed the effects of hormones on the callus growth. Callus formation was obtained from root tips in media supplemented with different combinations and concentrations of hormone. According to [17-20] callus formation was obtained if the concentration of auxin and cytokinin was the same. But, actually this statement was suitable only for certain species. For Brassica olerace var italica callus also obtained from media supplemented with different concentrations of auxin and cytokinin [21-24].

Table 3. Effects of different combination of hormones on fresh weight of callus produced from broccoli root tips

BAP	IAA	IBA	Callus weight (g)
1.0	0.25	0.25	-
	0.5	0.5	-
	1.0	1.0	1.3±0.09
	1.5	1.5	3.5±0.12
	2.0	2.0	2.3±0.25
	2.5	2.5	-
	0.25	0.25	-
1.5	0.5	0.5	
	1.0	1.0	2.27±0.2
	1.5	1.5	2.5±0.1
	2.0	2.0	2.4±0.01
	2.5	2.5	-
2.0	0.25	0.25	-
	0.5	0.5	-
	1.0	1.0	1.8±0.04
	1.5	1.5	2.18±0.06
	2.0	2.0	2.15±0.05
	2.5	2.5	-
2.5	0.25	0.25	-
	0.5	0.5	-
	1.0	1.0	1.85±0.04
	1.5	1.5	1.85±0.05
	2.0	2.0	1.75±0.04
	2.5	2.5	-
			-

Callus produced per leaves explant, Average ± SE of 10 replicates

4. CONCLUSION

The best medium for callus proliferation of broccoli was MS basal medium supplemented with 1.5 mg/l IAA, IBA combined with 1.0 BAP using root tips. For roots formation, the better media were MS basal medium supplemented with the concentration of IAA 0.25-2.5 and IBA 0.25-2.5 mg/l combined with 1.5-2.5 mg/l BAP compared to other combination of concentrations.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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