RESEARCH ARTICLE

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A study on bitter gourd (*Momordica charantia* L.) callogenesis optimization based on hormone balance and explant types

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Abstract

Bitter gourd known as *Momordica charantia* L. is an important summer vegetable, belongs to the *Cucurbitaceae* family, grown in tropical areas in the world. Due to its fruits being rich in vitamins, minerals and good dietary fibers, the bitter gourd has many health-protective and pharmacological properties. The goal of the current study was to comparatively assess the indirect regeneration capability using *in vivo* leaf, internodal, and petiole explants of bitter gourd. To serve the purpose Murashige and Skoog (MS) media augmented with various concentrations of auxin and cytokinin for callus formation potent. The study was conducted by evaluating the selection of various auxin and cytokinin concentrations and combinations in MS basic medium. The rate of explant development decreased as cytokinin concentration increased. Although the lowest cytokinin concentration utilized in this study (1.0 mg L^{-1} BAP), it had a beneficial impact on explant development. When compared to the auxin NAA, the auxins IAA and 2,4-D were more beneficial on calli weights. It has been well proven that calli cannot be produced without the presence and balance of plant growth regulators. Experimental results demonstrated that callogenesis of bitter gourd from different explants might be successfully and effectively utilized in cell suspension cultures, genetic transformation, and callogenesis may also be adaptable to other species that are closely related.

Keywords: Bitter Melon, Calli Formation, Internode, Leaf, Petiole

INTRODUCTION

The bitter melon, commonly known as the bitter gourd, belongs to the *Cucurbitaceae* family and a highly nutritious food, due to its high level of bioactive compounds with therapeutic uses. The bitter gourd plant is particularly rich in antioxidants, peptides, alkaloids, glycosides, vitamins A, B, and C, flavonoids, beta-carotene, and lutein. It has a low carbohydrate and fat content as well as few calories (Alina et al., 2016). *Momordica charantia* L. is used in folk healthcare to treat lung and digestive disorders as well as hepatitis, cancer, diabetes, HIV/AIDS, contraceptives, and viral infections (Alexandra and Dorica, 2010; Jin et al., 2019).

It is known that studies to reveal valuable bioactive components that are desired to be obtained from medicinally valuable plants and to increase existing components can support traditional agriculture on industrial scale, thanks to biotechnological approaches. The callus culture technique is an efficient method used for the production of secondary metabolites, which is a specific issue. Of course, as in every tissue culture technique, success in the callus culture

technique depends on some certain specific parameters. Explant source, type of explant, a basic nutrient medium used, type and concentration of carbon source, type, concentration, and combinations of various plant growth regulators such as auxin and cytokinin are the main factors affecting the potential success of callus cultures (Tariq et al., 2014). Although the callus culture technique is an efficient way to produce valuable bioactive components, there is a need to develop an effective callus formation system crop-specific in order to reduce the application cost of the techniques while increasing the yield and quality of the products.

Studies have been carried out on micropropagation, organogenesis and somatic embryogenesis of *M. charantia* L. by using various explants such as cotyledon, leaf, nodal, shoot tip, stem, root fragments by some researchers until today (Huda and Sikdar, 2006; Munsur et al., 2009; Paul et al., 2009; Thiruvengadam et al., 2010; Tang et al., 2011). However, callus and suspension culture studies have not been extensively conducted. In terms of genetic transformation studies and studies in the field of genetic engineering, callus and cell suspension cultures are needed effectively, but there is a need to increase the number of studies to be conducted in this field, since there is no sufficient and detailed research in the literature on *M. charantia* L. callus cultures (Thiruvengadam et al., 2006; Sultana and Rahman, 2012; Alina et al., 2016).

The current study aimed to organize and efficient callus culture protocol for bitter gourd by using three different explant types, namely leaf, internode, and petiole, and various plant growth regulator concentrations and combinations by evaluating the development of explants, and weights of formed calli.

MATERIALS AND METHODS

Plant cultivation and preparation of explants

For the callogenesis process, bitter gourds that were firm, ripe, uniform in color and appearance, and free of any flaws were harvested from a private garden in Antalya (36°53'10.1" N 30°45'23.4" E), Turkey. Bitter gourds, which were transferred to Akdeniz University, Faculty of Agriculture, Department of Horticulture, were washed, and cleaned, and then seeds were separated. The bitter gourd seeds at the same size, appearance, and maturity, were used (Figure 1). The three seeds were sown in plastic pots (13 x 35 cm size) filled with a 2:1 peat: perlite mortar mixture ratio.

After 30 days from seed sowing, while the bitter gourd plants were at the four-five leafy stage, explants of leaf, internodes, and petioles were removed and taken to the laboratory (see Figure 2). To serve the surface sterilization process, *in vivo* leaf, internode, and petiole explants were immersed in a 15% sodium hypochlorite solution (5% active substance) for ten minutes, and then rinsed three times with sterile distilled water.

Figure 1. Bitter gourd seeds

Figure 2. Surface sterilization (a), culturing the bitter gourd explants (b, c, d)

Media preparation, culture conditions, and setting up callus cultures

To see the calli development in bitter gourd, 19 different media (which also include a control medium) combinations were used in the present study (Table 1). The basic media was Murashige and Skoog medium (MS, 1962), supplemented with different concentrations and mixtures of BAP, NAA, 2,4-D, and IAA. All of the explants used in the study were cultured at a specific size (0.5 to 1.0 cm), and maintained under growth chamber settings at temperature of 24±2 °C, a photoperiod of 16 hours of light and 8 hours of darkness, and a light intensity of 3000 $E.m^{-2}.s^{-1}$. Developed explants and calli were subjected to 3 subcultures with 30 days intervals.

Evaluated parameters

Developments of leaf, internode, and petiole explants (%), and formed callus fresh weights (g) were evaluated

Table 1. Combinations of Media Utilized for Callogenesis Process

Media No	Media Combinations						
	MS (g L^{-1})	BAP (mg L^{-1})	NAA (mg L^{-1})	2,4-D (mg L^{-1})	IAA (mg L^{-1})	Sucrose (g L^{-1})	Agar (g L^{-1})
1 (cont.)	4.4	-	-	-	-	30	6
2	4.4	1.0	0.5	-	-	30	6
3	4.4	1.0	1.0	-	-	30	6
4	4.4	1.0	-	-	0.5	30	6
5	4.4	1.0	-	-	1.0	30	6
6	4.4	1.0	-	0.5	-	30	6
7	4.4	1.0	-	1.0	-	30	6
8	4.4	2.5	0.5	-	-	30	6
9	4.4	2.5	1.0	-	-	30	6
10	4.4	2.5	-	-	0.5	30	6
11	4.4	2.5	-	-	1.0	30	6
12	4.4	2.5	-	0.5	-	30	6
13	4.4	2.5	-	1.0	-	30	6
14	4.4	5.0	0.5	-	-	30	6
15	4.4	5.0	1.0	-	-	30	6
16	4.4	5.0	-	-	0.5	30	6
17	4.4	5.0	-	-	1.0	30	6
18	4.4	5.0	-	0.5	-	30	6
19	4.4	5.0	-	1.0	-	30	6

after each subculture. Three subcultures were carried out to promote the weight of the calli obtained. To determine the developments of leaf, internode, and petiole explants (%) the following equation was separately used for each.

Leaf, internode, and petiole explants development (%) = (number of developed leaf, internode, and petiole explants/ number of total cultured leaf, internode, and petiole explants) × 100

Statistical analysis

The experiment of callogenesis process was carried out as 3 replicates and 3 subcultures were conducted for each explant type. Four petri dishes with 10 explants of each leaf, internode, and petiole explant parts were utilized in each repetition. At the end of 3 subcultures for each explant type, the mean values were evaluated, and obtained result data were subjected to Duncan test at $p < 0.05$ level by using SPSS (Version 17; Chicago, IL, USA) statistics software.

RESULTS AND DISCUSSION

Evaluations of explant types

In terms of both explant development and produced calli weights, the results of experiments definitely indicated that there was a statistically important distinction among three different types of explants. Regarding explants types, in comparison, the responses of leaf explants were better than internode and petiole explants (Table 2).

Evaluations of media combinations

Regarding the percentages of explants development, there were statistically important distinctions among various media combinations (Table 3). The medium number 6 which was augmented with 1.0 mg L^{-1} BAP + 0.5 mg L^{-1} 2,4-D, was the best among all 19 media combinations regarding percentages of explants development, while the control medium and medium number 18 (5.0 mg L^{-1} BAP + 0.5 mg L^{-1} 2,4-D) had no positive effects on explant developments.

Regarding the calli weights, statistically important variations were found among media combinations (Table 3). MS basic medium supplemented with 2.5 mg L^{-1} BAP + 0.5 mg L^{-1} IAA (medium No. 10) and 1.0 mg L^{-1} BAP + 0.5 mg L^{-1} 2,4-D (medium No. 6) were the best when the effects of different media combinations on explant development. On the other hand, there were no favorable impacts of control medium or medium number 7 (1.0 mg L^{-1} BAP + 1.0 mg L^{-1} 2,4-D) on calli weights.

It is widely known that several factors influence the synthesis and accumulation of secondary metabolites in explants cultivated *in vitro*, as well as the callogenesis process. Genotype, different types of explants, composition, and combination of media, carbohydrate supply, plant growth regulators types, concentrations and combinations, and culture conditions are among the parameters that have been found to be crucial (Siatka, 2019).

Table 2. Explant Development (%) and Calli Weights Based on Explant Types

Explants	Percentages of explants development	Mean values of formed calli weights
Leaves	$65.56^{c} \pm 21.31$	$0.21^{c} \pm 0.13$
Internodes	$19.97^{b} \pm 15.87$	$0.12^{b} \pm 0.10$
Petioles	$5.64^{a} \pm 7.01$	$0.06^{a} \pm 0.11$

*According to the Duncan multiple comparison results, the difference between the means with the same letter is insignificant.

Table 3. Explant Development (%) and Calli Weights Based on Media Combinations

Media	Percentages of explants development	Mean values of formed calli weights
1	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
2	$32.41^{bc*} \pm 18.33$	$0.18^{cde*} \pm 0.07$
3	$29.45^{abc*} \pm 20.87$	$0.14^{b-e*} \pm 0.14$
4	$35.00^{bc*} \pm 30.00$	$0.16^{b-e*} \pm 0.13$
5	$40.47^{bc*} \pm 18.04$	$0.12^{b-e*} \pm 0.12$
6	$50.28^{c} \pm 34.53$	$0.21^{e} \pm 0.10$
7	$21.21^{abc*} \pm 2.95$	$0.05^{ab*} \pm 0.11$
8	$38.33^{bc*} \pm 29.04$	$0.20^{de*} \pm 0.12$
9	$26.30^{abc*} \pm 47.17$	$0.12^{a-e*} \pm 0.16$
10	$37.69^{bc*} \pm 36.11$	$0.22^{e} \pm 0.13$
11	$25.38^{abc*} \pm 24.79$	$0.14^{b-e*} \pm 0.14$
12	$25.00^{abc*} \pm 43.30$	$0.09^{a-d*} \pm 0.14$
13	$42.41^{bc*} \pm 33.05$	$0.13^{b-e*} \pm 0.13$
14	$38.33^{bc*} \pm 42.49$	$0.14^{b-e*} \pm 0.14$
15	$29.17^{abc*} \pm 41.23$	$0.14^{b-e*} \pm 0.14$
16	$24.17^{abc*} \pm 47.63$	$0.09^{a-e*} \pm 0.14$
17	$26.95^{abc*} \pm 45.48$	$0.12^{b-e*} \pm 0.15$
18	$18.05^{ab*} \pm 31.85$	$0.07^{a-d*} \pm 0.11$
19	$24.26^{abc*} \pm 41.50$	$0.07^{abc*} \pm 0.11$

*According to the Duncan multiple comparison results, the difference between the means with the same letter is insignificant.

Thiruvengadam et al. (2006) and Sultana and Rahman (2012) reported a positive trend about calli inducing using leaf explants of bitter gourd on MS basic medium fortified with 1.0 mg L^{-1} 2,4-D. Paul et al. (2009) obtained the best bitter gourd calli formation on MS media fortified with 0.5 mg L^{-1} NAA and 5.0 mg L^{-1} BAP, in other words, relatively high cytokinin concentration was necessity for calli formation in their study. On the other hand, in the present study, the best explants that developed calli and showed best explant development were obtained in MS media suplemented with low BAP (1.0 mg L^{-1}) and low 2,4-D (0.5 mg L^{-1}) concentrations. Also, the same media combination above stated along with a relatively high BAP (2.5 mg L^{-1}) and low IAA (0.5 mg L^{-1}) combinations were found to be effective on calli weights. Callus induction for *Momordica cymbalaria* was reported by Jeyaprakasam et al. (2021). Researchers cultured explants (leaf, root tubers, internode, fruit) of *Momordica cymbalaria* for callus induction in MS medium supplemented with different growth regulators at various concentrations. It was demonstrated that callus was induced from leaf and internode explants when MS medium was supplemented with 1 mg L^{-1} and 5 mg L^{-1} of 2,4-D, respectively. Chung et al. (2016) reported that the highest callus frequency occurred when culturing *Momordica dioica* leaf explants with 1 mg L^{-1} NAA and 0.5 mg L^{-1} TDZ. It was also demonstrated that TDZ combined with IAA or 2,4-D, both of which induced less callogenesis than the NAA plus TDZ combination.

As reported by numerous researches on different plant types, the callogenesis process could not be conducted in a medium without plant growth regulators of auxin and cytokinin (Elaleem et al., 2009; Ozsan and Onus, 2020). However, media combination should be supplemented with appropriate concentration and combination of auxin and cytokinin to trigger calli formation as well as explant types (Lestari et al., 2019). Results of current study and results of previous studies indicated the importance of precense of auxins and cytokinins as well as the balance between auxin and cytokinin concentration for calli formation (Baharan et al., 2015; Efferth, 2019).

CONCLUSION

The increase in cytokinin amount led to a decrease in the explant development rates, while the lowest cytokinin amount (1.0 mg L^{-1} BAP) which was used in the current study had a positive effect on explant development. The

auxins IAA and 2,4-D at 0.5 mg L^{-1} concentration were favorable to calli weights compared with the NAA as auxin source. The findings of the present study also clearly revealed that there were differences among explant types regarding callus induction and weights and leaf explants came into prominence. It is believed that more diverse media combinations should be optimized based on the explant types used for callus formation in future research. It is assumed that the findings of the present study may serve as a guide for scientists working on bitter gourd breeding and biopharmaceuticals employing callus and cell suspension cultures.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. Both authors read and approved the final manuscript. Both authors verify that the text, figures, and tables are original and that they have not been published before.

Ethics committee approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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