In Vitro Sterilization and Shoot Induction of Fig (*Ficus carica* L.) Using MS Containing GA_3 Medium Supplemented with BAP and NAA

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Abstract The purpose of this research was to obtain the proper sterilization method for fig (*Ficus carica* L.) shoot and determine the best combination of BAP and NAA for in vitro shoot induction of fig. The sterilization research was arranged in completely randomized design (CRD), a single factor with five treatments. The treatments tested were dipping in NaClO (0.1, 5% for 7 min), fungicide and bactericide 4 g/L (5 min, 30 min, 1 h, 2 h, 3 h). Each treatment was repeated three times. Parameters measured were the percentage of browning explants, the percentage of contaminated explants and the percentage of survive explants. Shoots induction research was arranged in completely randomized design (CRD), a single factor with seven treatments. The treatments tested were the addition of BAP (0, 2, 4, 6 mg/L) and NAA (0, 0.5, 1 mg/L) to the MS medium containing GA_3. Each treatment was repeated six times. Parameters measured were the percentage of contaminated explants, the percentage of survive explants, percentage of shoot formation, percentage of browning explants, number of shoots, shoot height, number of leaves and percentage of rooted explants. The best method for fig shoot sterilization was dipping explants in NaClO10%, 5′ + NaClO 5%, 7′ + fungicide and bactericide 4 g/L for 3 h with the percentage of survival explants 100% during 30 days of observation. The addition of 2 mg/L BAP and 0.5 mg/L NAA into MS containing GA_3 medium was favorable to produce among the highest percentage of fig shoot formation (33.33%) and the browning explants (0.00%).

Keywords Fig (*Ficus carica* L.) · Sterilization · Shoot induction · BAP (6-benzylaminopurine) · NAA (a-naphthalene acetic acid) · GA_3 (gibberellin acid)

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1 Introduction

Fig (*Ficus carica* L.) is known as relatives of banyan tree. This plant is widely grown in coastal areas Balkans to Afghanistan, and then developed in Australia, Chile, Argentina, the USA, Middle Eastern countries and Mediterranean region. The Global Diabetes Community [1] stated that the fig leaf is known as diabetes drugs in Spain and southwestern Europe. Fig fruit contains many nutrients such as carbohydrates, proteins, vitamins, minerals, fiber and others. Fig fruit also contains high dietary fiber. Each 100 g of dried fig contains 10.95 g fiber in which this concentration is higher than those in apple and orange fruit, i.e., 3.33 and 3.4 g. Fig also contains unsaturated fatty acids such as omega-3 23.87 + 6.27%, omega-6 23.04 + 0.48% and omega-9 19.72 + 1.07% that function to prevent coronary heart disease [2]. Each 100 g or dried figs contain 180.75 mg calcium, 75.75 mg magnesium, 759.75 mg potassium and 2.26 mg iron [3].

Based on its benefit and it is still a rare fruit in Indonesia, the tin has a great opportunity to be cultivated. Fig trees are still planted in some areas in Indonesia, especially in Java [4]. Therefore, a technique such as in vitro culture to multiply large quantities of shoots in a short amount of time is needed. In vitro culture is influenced by the use of plant growth regulator as a driver of growth and development of explants, such as naphthalene acetic acid and Benzylaminopurine. Those NAA and BAP can be added alone or together into medium, and the explants morphogenesis will be depend on interaction of both growth regulators [5]. The objective of this study was to obtain proper sterilization method and to determine the best concentration of BAP and NAA in fig shoots induction in the MS medium containing GA_3.

2 Materials and Methods

The experiment was conducted in In Vitro Culture Laboratory, Faculty of Agriculture, University Muhammadiyah Yogyakarta. The materials used in this research were fig shoot explants, plant growth regulators such as BAP, NAA and GA_3, sterilization compounds, i.e., bactericide, fungicide, NaClO 5%, ethanol 70%, detergent, iodine and vitamin C. The basic culture medium used in this study was Murashige and Skoog (MS) medium.

This study consisted of two experiments, first was the optimization of sterilization, and second was the effect of plant growth regulators on the induction of fig shoots in MS medium. The treatments in this research are: types, concentration and dipping time of sterilization compounds at first experiment, whereas the concentration of BAP (0, 2, 4 and 6 mg/L) and NAA (0, 5 and 1 mg/L) was used at second experiment.

2.1 Optimization of Sterilization

In this study, the variables were the concentration of sterilization compounds and dipping time in the sterilization liquid as shown in Table 1. Experimental units were arranged in completely randomized design (CRD) one factor. Each treatment combination was replicated five times.

2.2 Shoot Induction

Shoots induction research was arranged in completely randomized design (CRD), a single factor with seven treatments. The treatments tested were the addition of BAP (0, 2, 4, 6 mg/L) and NAA (0, 0.5, 1 mg/L) to the MS medium containing GA_3. Each treatment was repeated six times. Parameters measured were the percentage of contaminated explants, the percentage of survives explants, percentage of shoot formation, percentage of browning explants, number of shoots, shoot height, number of leaves and percentage of rooted explants.

The treatments were as follows

A = Medium MS + BAP 0 mg/L + NAA 0 mg/L + GA_3 0.5 mg/L
B = Medium MS + BAP 2 mg/L + NAA 0.5 mg/L + GA_3 0.5 mg/L
C = Medium MS + BAP 4 mg/L + NAA 0.5 mg/L + GA_3 0.5 mg/L
D = Medium MS + BAP 6 mg/L + NAA 0.5 mg/L + GA_3 0.5 mg/L
E = Medium MS + BAP 2 mg/L + NAA 1 mg/L + GA_3 0.5 mg/L
F = Medium MS + BAP 4 mg/L + NAA 1 mg/L + GA_3 0.5 mg/L
G = Medium MS + BAP 6 mg/L + NAA 1 mg/L + GA_3 0.5 mg/L

3 Result and Discussion

3.1 Optimization of Sterilization

Explants sterility is one of the factors that influence the success of in vitro culture; therefore, the appropriate method of sterilization is very important to provide sterile explants. The result of sterilization observation was provided in Table 2.

Percentage of Browning Explant

The highest percentage of browning (100%) was obtained on treatment C which used NaClO 10 and 5%, respectively, then followed by the addition of fungicide 4 g/L and bactericide 4 g/L for 1 h (Table 2). Otherwise, there is no explants browning which achieved on treatment NaClO 1% 5′ + NaClO 0.1% 7′ + fungicide and bactericide 4 g/L for 30′ (B), NaClO 10%, 5′ + NaClO 5% 7′ + fungicide

Table 1 Type of sterilization compounds and their concentration, dipping time in sterilization liquid used for optimization of shoot fig sterilization

Treatment	Step 1		Step 2		Step 3		Step 4		Step 5		Step 6	
	Compound	Time	Compound	Time	Compound	Time	Compound	Time	Compound	Time	Compound	Amount
A	NaClO 1% + liquid detergent 2 drops	5′	Fungicide 4 g/L + bactericide 4 g/L	5′	Ethanol 70%	30″	NaClO 0.1%	7′	Vitamin C 0.1%	4–5 x	Iodine	5 drops
B	NaClO 1% + liquid detergent 2 drops	5′	Fungicide 4 g/L + bactericide 4 g/L	30′	Ethanol 70%	30″	NaClO 0.1%	7′	Vitamin C 0.1%	4–5 x	Iodine	5 drops
C	NaClO 10% + liquid detergent 2 drops	5′	Fungicide 4 g/L + bactericide 4 g/L	1 h	Ethanol 70%	30″	NaClO 5%	7′	Vitamin C 0.1%	4–5 x	Iodine	5 drops
D	NaClO 10% + liquid detergent 2 drops	5′	Fungicide 4 g/L + bactericide 4 g/L	2 h	Ethanol 70%	30″	NaClO 5%	7′	Vitamin C 0.1%	4–5 x	Iodine	5 drops
E	NaClO 10% + liquid detergent 2 drops	5′	Fungicide 4 g/L + bactericide 4 g/L	3 h	Ethanol 70%	30″	NaClO 5%	7′	Vitamin C 0.1%	4–5 x	Iodine	5 drops

Table 2 Effect of sterilization method on explants sterility after 30 days of planting

Treatment	Percentage of browning explants(%)	Percentage of contaminated explants (%)	Contaminants	Day of contamination	Percentage of survive explants (%)
A	66.67	100	Fungi	14.33	0
B	0	100	Fungi, bacteria	7.33	0
C	100	100	Bacteria	12	0
D	0	66.67	Bacteria	3	33.33
E	0	0	–	–	100

and bactericide 4 g/L for 2 h (D) and the treatment NaClO 10%, 5′ + NaClO 5% 7′ + fungicide and bactericide 4 g/L for 3 h (E). Browning occurs due to the high content of phenolic compounds which are oxidized when cells are injured, consequently the isolated tissue turn into brown and failure to thrive [5].

Percentage of Contaminated Explant

The result of sterilization showed that among five treatments tested, four of them caused explants contamination in different time (Table 2). It can be explained that dipping time in sterilization compound was too short and microorganism-infected explants may be used. The lowest contaminated explants (0%) was observed on treatment E which explants sterilized in fungicide 4 g/L and bactericide 4 g/L for 3 h. This result showed that dipping explants in fungicide and bactericide for 3 h were able to eliminate fungi and bacteria.

Percentage of Survive Explant

The effect of sterilization compound and dipping time showed that the longest dipping (3 h) in fungicide and bactericide resulted the highest percentage of survive explants (100%). Fungicide and bactericide used in this study is systemic which their active ingredients penetrate into plant tissue as stated by Wudianto [6]. So that, either fungicide or bactericide eliminates the fungi and the bacteria inside the plant tissue. This study showed that the longest dipping in fungicide and bactericide (treatment E) gave the lowest percentage of browning explants (0%), the lowest percentage of contaminated explants (0%) and the highest percentage of survive explants (100%), therefore, treatment E was chosen as sterilization method in shoot induction of Fig.

3.2 Shoot Induction of Fig

Shoot induction of Fig was strongly influenced by the survive explants and growth rate. The effect of BAP and NAA on shoot induction of Fig was shown in Table 3.

Table 3 Effect of BAP and NAA on the percentage of contaminated explants, the percentage of survive explants, the percentage of callus formation, the percentage of shoot formation and the percentage of browning explants after 8 weeks of culture

Treatment	Percentage of contaminated explants (%)	Percentage of survive explants (%)	Percentage of browning explants (%)	Percentage of shoot formation (%)
0 mg/L BAP + 0 mg/L NAA	40.48	50.00	16.67	0.00
2 mg/L BAP + 0.5 mg/L NAA		50.00	0.00	33.33
4 mg/L BAP + 0.5 mg/L NAA		66.67	33.33	16.67
6 mg/L BAP + 0.5 mg/L NAA		66.67	16.67	0.00
2 mg/L BAP + 1 mg/L NAA		66.67	16.67	16.67
4 mg/L BAP + 1 mg/L NAA		50.00	16.67	16.67
6 mg/L BAP + 1 mg/L NAA		66.67	16.67	16.67

Percentage of Contaminated Explants

To survive and grow properly, in vitro plant cultures need to be largely free from pest, fungi and bacterial infections. Results showed that the percentage of contaminated explants was 40.48% (Table 3) which caused by fungi and bacteria. The contamination was occurred in week 1 until 6. This contamination may be caused by endogenous microbial inside plant tissues. This was in agreement with Fahmadi [7] who stated that endogenous contamination derived from microbial contamination in plant tissues. The non-sterile explants which shipped from outstation may cause contamination. Microorganisms penetrate into explants via the water and the air in the shipping.

Percentage of Browning Explants

The results in Table 3 showed that 4 mg/L BAP + 0.5 mg/L NAA produced the highest percentage of browning explants (33.33%, Fig. 1a), otherwise 2 mg/L BAP + 0.5 mg/L NAA produced the lowest percentage of browning explants (0%, Fig. 1b). The browning explants may caused by the sap released cut explants, which continuously released hydroxyphenol. This was in agreement with Tabiyeh et al. [12] whereby in vitro browning caused by increasing of phenolic exudate from explants which then followed by their oxidation. Fig is perennial plant which containing sap; therefore, cutting explants induce the oxidized sap released from the shoots.

Percentage of Shoot Formation

Shoots began to grow at day 25 in the nodes. The results showed that treatment 2 mg/L BAP + 0.5 mg/L NAA was among the concentration which induced the highest percentage of shoot formation (33.3%, Fig. 2b) which was not significantly different with the percentage of shoot formation obtained on 4 mg/L

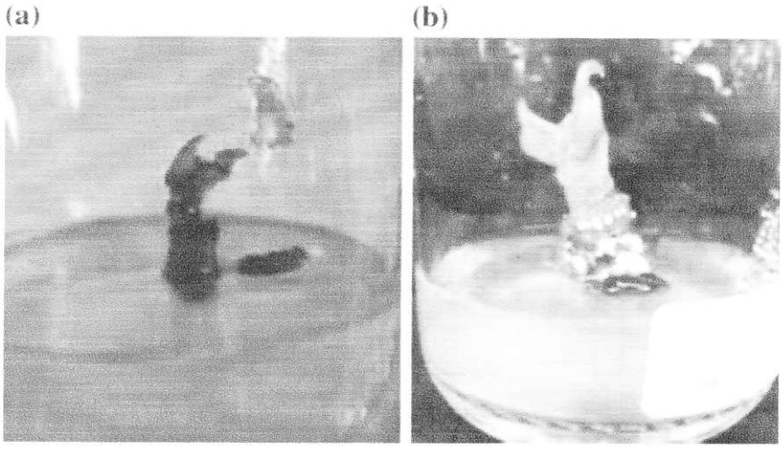

Fig. 1 Browning explants cultured on MS + 4 mg/L BAP + 0.5 mg/L NAA (**a**) and non-browning explants cultured on MS + 2 mg/L BAP + 0.5 mg/L NAA (**b**)

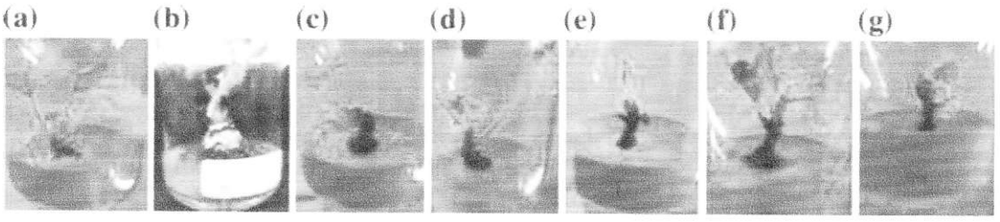

Fig. 2 Explants cultured on MS containing GA_3 + 2 mg/L BAP + 0.5 mg/L NAA after 7 weeks of culture

Table 4 Effect of BAP dan NAA on number of Fig shoots, shoot height, number of leaves and percentage of rooted explants after 8 weeks of culture

Treatment	Number of shoot	Shoot height (cm)	Number of leaves	Percentage of rooted explants (%)
0 mg/L BAP + 0 mg/L NAA	0.00	0.27	1.67	0.00
2 mg/L BAP + 0.5 mg/L NAA	0.67	1.27	1.33	0.00
4 mg/L BAP + 0.5 mg/L NAA	0.25	1.08	2.50	0.00
6 mg/L BAP + 0.5 mg/L NAA	0.00	0.55	1.75	0.00
2 mg/L BAP + 1 mg/L NAA	0.25	1.43	1.50	0.00
4 mg/L BAP + 1 mg/L NAA	0.33	1.33	2.67	0.00
6 mg/L BAP + 1 mg/L NAA	0.25	0.63	0.63	0.00

BAP + 0.5 mg/L NAA (Fig. 2c), 2 mg/L BAP + 1 mg/L NAA (Fig. 2e), 4 mg/L BAP + 1 mg/L NAA (Fig. 2f) and 6 mg/L BAP + 1 mg/L NAA (Fig. 2g) (16.67%). The results also showed that all treatment-induced shoots except those explants cultured on MS containing GA_3 medium without BAP + NAA (control, Fig. 2a) and MS containing GA_3 + 6 mg/L BAP + 0.5 mg/L NAA (Fig. 2d). This could be explained that medium without BAP did not able to induce the adventitious shoots. This result was in agreement with the study reported by Julianti [8], whereby 0.1 mg/L NAA and 2.5 mg/L BAP induced the highest shoots formation (12 shoots) in *Aquilaria malaccensis* Lamk. However, treatment 6 mg/L BAP + 0.5 mg/L NAA was not able to induce shoot formation, because explants may had not responded to BAP and NAA supplemented into MS medium.

Number of Shoot

The results showed that there was no significant difference on the number of shoots produced by the various concentration of BAP and NAA supplemented into MS containing GA_3 medium (Table 4). The treatment 2 mg/L BAP + 0.5 mg/L NAA produced 0.67 shoots. This result contrasted with results reported by Kumar et al. [9] which stated that 2 mg/L BAP induced 4.8 shoots/explants and the average of shoot height achieved was 5.7 cm. The growth of shoots on the different medium may be caused by the interaction between BAP and NAA in stimulating growth and differentiation of explants. Wareing and Phillips [10] similarly demonstrated

cytokinin induce plant cells division and interact with auxin to lead the differentiation of cells. The higher concentration of cytokinin than auxin may stimulate shoots and leaves formation.

Shoot Height and Number of Leaves

The results showed that there was no significant difference on the shoot height and number of leaves produced by the various concentration of BAP and NAA supplemented into MS containing GA_3 medium (Table 4). The increasing concentration of BAP showed the decreasing of shoot height as shown in Table 4. The results above were supported by George and Sherrington [5] who stated that the effect of cytokinin in tissue cultures is to inhibit apical dominance and encourage the growth of axillary buds.

Percentage of Rooted Explants

The results showed that all treatments did not produce roots after 8 weeks of culture. This study contrasted with results reported by Kumar et al. [9] which stated that medium MS + 2 mg/L NAA was able to induce 45.2 ± 1.1% fig roots. These different results may be explained that the NAA concentration supplemented into MS medium was too low; therefore, the explants did not have enough auxin to produce the adventitious roots. This result was in agreement with those reported by Armini et al. [11] who stated that in vitro cultured explants require auxin for inducing roots. On the other hand, the growth of adventitious roots required longer observation than 8 weeks to induce explants in producing roots.

4 Conclusion

The best method for fig shoot sterilization was dipping explants in NaClO10%, 5′ + NaClO 5%, 7′ + fungicide and bactericide 4 g/L for 3 h with the percentage of survival explants 100% during 30 days of observation. The addition of 2 mg/L BAP and 0.5 mg/L NAA into MS containing GA_3 medium was favorable to produce among the highest percentage of fig shoot formation (33.33%) and the browning explants (0.00%).

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