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Research Article

Micropropagation of Male and Female Trees of *Andaleh* (*Morus macroura* Miq.) through In-vitro Culture using Several Compositions of Basal Medium

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Abstract

Background and Objective: *Andaleh* is the local name of *Morus macroura* Miq. in West Sumatra, Indonesia. Nowadays, this dioeciously species is in endangered situation. The aim of the research is to find out the appropriate combination of plant growth regulator to induce shoot multiplication of explants from male and female trees of *andaleh*. The plantlets from this research will be used in the next future to conserve this endangered species in vitro and in vivo, especially in preparing parental material in breeding program. **Materials and Methods:** Young buds from male and female trees were used as an explants in basal medium Murashige and Skoog supplemented with BAP (0.5, 1.0, 1.5 and 2.0 mg.L^{-1}) in combination with NAA (1.0 mg.L^{-1} for each). **Results:** The frequency of bud break was 50 % in MS medium supplemented with BAP (0.5 mg.L^{-1}) and NAA 1.0 mg.L^{-1} for both source of explants (female and female trees of *andaleh*) after 3 weeks of culture**.** Generally, the number of shoot induction was very low. On the other hand, the rate of callus formation was high (100%) in the highest BAP concentration (2.0 mg.L^{-1}).

Key words: *Andaleh*, dioecious, endanger, in-vitro, *Morus*.

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Introduction

Andaleh belongs to the *Moraceae* family and to the genus *Morus* (Miquel,1862). In his book "*Sumatra III Zijne Plantenwereled*", he wrote that the specimen used to identify the plant came from Batang Baroes, Sumatra and named it *Morus macroura*. This species has the same name with Himalayan mulberry, but with a little different especially in the fruit set. Based on this condition, Syamsuardi (2015) suggested putting var. *macroura* along with the name given by Miquel. So, he suggests the scientific name of *andaleh* is *Morus macroura* Miq. var. *macroura*. In the past, the wood from these trees was used in the traditional house of Minangkabau, handicraft and cabinet work. With the reduction in number of individuals and populations of *Andaleh*, it was put on the list of rare plants species in Indonesia (Mogea *et al.*, 2001).

As a family of mulberry, *andaleh* has the great economic value. Several countries have already made extensive efforts to collect and conserve mulberry (Tikader and Vijayan, 2010). Vijayan *et al.* (2011) explained that the conservation strategies can be divided into *in situ* conservation, *ex situ* conservation, *in vitro* conservation and DNA banking. In order to support *in vitro* conservation, first we have to establish the protocol of *in vitro* clonal propagation.

In vitro establishment of nodal segments collected from mature trees of different species of Morus has been reported (Narayan *et al.*, 1989; Pattnaik and Cland, 1997; Vijayan *et al.*, 2000; Anis *et al.*, 2003; Thomas, 2003; Zaki *et al.*, 2011; Akram and Aftab, 2012). However, no significant prior information in the contemporary literature is available on in-vitro micropropagation of male and female trees of *andaleh* (*M. macroura* Miq. var. *macroura*). The present study was therefore conducted with the aim to find out the suitable protocol to induce shoot multiplication from nodal explants of male and female trees of *andaleh.*

Materials and Methods

Plant Material and Culture Conditions

Young buds from male and female *andaleh* trees were collected and surface sterilized. Each bud was prepared and thoroughly rinsed with running tap water for 15 minutes, 70 % ethanol for 30 seconds and 15 % commercial bleach (v/v) for 15 minutes and then rinsed with sterile distilled water 3 to 5 times. Dead tissues after surface-sterilization on both ends of the nodal explants were trimmed and 1 cm long nodal segments were inoculated on the culture medium for shoot induction. MS medium (Murashige and Skoog, 1962), added 30 g L^{-1} sucrose, 8 g L^{-1} agar, supplemented with BAP (0.5, 1.0, 1.5 and 2.0 mg L^{-1}) in combination with NAA (1.0 mg L^{-1} for each), pH = 5.7- 5.8 (adjusted by NaOH 1N or HCl 1N) was used for axillary bud and shoot initiation. The medium sterilized by autoclaving at 121 ^{o}C and 1 atm for 20 minutes. The data for bud break, number of shoot induction, callus formation, medium browning and number of days to bud break were recorded.

Data Analysis

Completely randomized design was used for the experiments. The data variance were analyzed using SPSS version 12.0 and the significance was then further tested using DNMRT (Duncan's New Multiple Range Test) with a $p<0.05$. The data were transformed if necessary using various formulas.

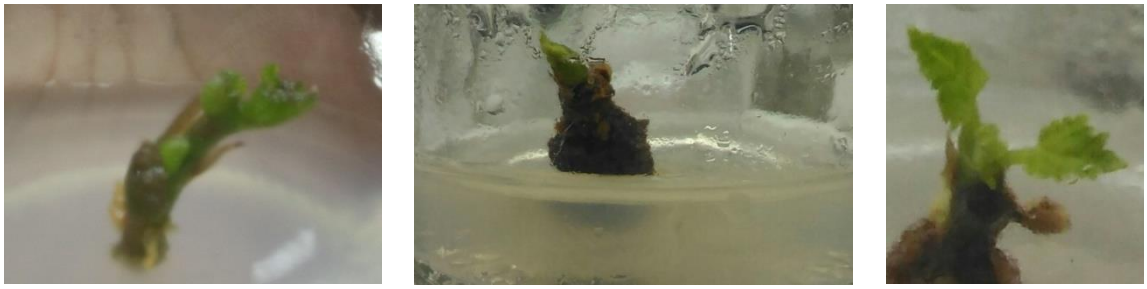
Results

Axillary Bud Break

The frequency of bud break was 50 % in MS medium supplemented with BAP (0.5 mg.L^{-1}) and NAA 1.0 mg L^{-1} (Table 1) for both source of explants (female and female trees of *andaleh*) after 3 weeks of culture. The effect of BAP concentration is different on female and male explants. In the female explants, increasing of BAP concentration was decrease the frequency of bud break and no bud break at the concentration of 2.0 mg L^{-1} BAP. On the other hand, in the male explants of *andaleh*, the frequency of bud break increase from 50 % (0.5 mg L^{-1} BAP) to 62.5 % (1.0 mg L^{-1} BAP) and after that the frequency was decreased.

Number of Days to Bud Break

After 5 days of in the MS medium supplemented with BAP and NAA the explants from male and female trees of *andaleh* was starting to break the buds (Table 1 and Fig. 1a). But, in the medium with 2.0 mg L^{-1} BAP, there was no bud break until the end of the research.



a **b** **c**

Figure 1. Bud break and shoot induction from the male and female *andaleh* explants in MS medium supplemented with BAP and NAA. (a) Bud break after 5 days. (b) Bud break of female. (c) Shootlet formation of male explant.

Number of Shoot Induction

The number of shoot induction was very low (Table 1). Only one and two shoots were induced by supplemented with BAP and NAA to MS medium. In higher concentration of BAP (2.0 mg L^{-1}) there was no shoot induced. On MS basal medium supplemented with BAP and NAA in low concentration, the shoot buds sprouted after 5 days incubation in culture room (Fig. 1a). The shoots obtained from male and female nodal explants of *andaleh* gave different growth and performance. The shoots from female trees did not elongated on the same medium up to 5 weeks (Fig.1b). On the other hand, the shoots from male trees were growing well (Fig. 1c).

Callus Formation

The rate of callus formation was generally increased by increasing BAP level and reach 100 % in 2.0 mg L^{-1} BAP (Table 1). In the female explants, the rate of callus formation increase from 37.5 % in 0.5 mg L^{-1} BAP to 50 % in 1.0 mg L^{-1} BAP and 67.5 % in 1.5 mg L^{-1} BAP. The rates of callus formation were relatively low in the male explants (37.5-40 %).

The development of callus is a common phenomenon during shoot induction. Callus will be growth at the shoot base or from aerial portion of nodal explants. In the present study, the callus formed in both parts (Fig. 2). Some of callus from nodal explants of male trees came from aerial portion (Fig. 2a) and from nodal explants of female trees came from the shoot base (Fig. 2b). Unfortunately, after 7 weeks in the incubation room, several of them turned brown and contaminated (Fig. 2c).

A **b** **c**

Figure 2. Callus formation from nodal explants of male and female trees of *andaleh*. (a) Callus formed from the aerial portion in nodal from male trees. (b) Callus formed from the shoot base in nodal from female trees. (c) Browning and death explant after callus formation.

Table 1. Effects of BAP and NAA on axillary bud break, shoot initiation and callus formation of male and female *andaleh* explants after 3 weeks of culture.

Source of explants	Combination of BAP and NAA (mg L^{-1})			
	0.5 BAP + 1.0 NAA	1.0 BAP + 1. 0 NAA	1.5 BAP + 1.0 NAA	2.0 BAP + 1.0 NAA
	Axillary bud break (%)			
Female	50.0	37.5	37.5	0.0
Male	50.0	62.5	37.5	0.0
	Number of bud breaking days			
Female	5	6	6	-
Male	6	5	5	-
	Number of emerged shoots			
Female	1	1	1	0
Male	1	2	1	0
	Callus formation (%)			
Female	37.5	50	67.5	100
Male	40	37.5	40	100

Minus (-) indicated there was no bud break.

Discussions

The present study indicated information about the prospect of MS basal medium supplemented with BAP and NAA for *in vitro* conservation of *M. macroura* Miq. var. *macroura*. The low concentrations of BAP with 1.0 mg L^{-1} NAA were suitable to induce shoot formation. Callus formation were relatively increasing if the concentrations of BAP higher than 2.0 mg L^{-1}.

Ohyama (1974) using axillary buds of *M. alba*, demonstrated for the first time that complete plants could be regenerated if explants are cultured on Murashige and Skoog (1962) medium supplemented with growth regulators. Shoot regeneration in mulberry is highly dependent on genotype, the type of explant, and the combination of plant growth regulators used in the culture medium (Feyissa *et al.*, 2005). In vitro propagation of mulberry has been reported using apical or axillary buds, or nodal explants (Benedetta *et al.*, 2007; Vijayan *et al.*, 2014), leaf explants (Kapur *et al.*, 2001), or shoot tips (Zaki *et al.*, 2011).

Shoots grew well when the culture medium contained cytokinin or cytokinin combination with auxins. Low concentration of auxin combined with high concentration of cytokinin was the best for shoot growth (Tejavathi and Gayathramma, 2005). Several studies also reported a combination of NAA and BAP to produce stronger shoots (Sita, 1982; Upadhaya and Chandra 1983). According to Lalitha *et al.* (2013), basal medium containing 2 mg L^{-1} of BAP was the best medium for shoot multiplication. Vijayan *et al.* (2000) observed the shoots growth on MS + BAP 2 mg L^{-1} and glucose medium. The highest propagation from shoots of *Morus* varieties was observed on MS medium with 2 mg L^{-1} BAPP+ 1mg.L^{-1} NAA.

In the case of *M. navigate*, winter dormant buds started sprouting after 12-25 days. In the case of BAP, the maximum rate of shoot induction (80 %) was observed with 1.0 mg L^{-1} BAP, followed by 0.5 mg L^{-1} BAP (66.7 %) or 1.5 mg L^{-1} BAP (60 %) after 14, 16 and 20 days length among various PGRs (Anis *et al.*, 2003; Chitra and Padmaja, 2005). The highest rates of shoot multiplication previously reported in *M. indicia* have been with 0.5 mg L^{-1} BAP (Chitra *et al.*, 2014) or 2.0 mg L^{-1} BAP (Lalitha *et al.*, 2013). However, it is also important to note that concentrations of BAP > 2.0 mg L^{-1} are inhibitory for shoot initiation and multiplication. The highest rooting percentage in mulberry was achieved on 0.5 MS medium. The length of roots decreased with higher concentrations of IBAP or NAA. Similarly, it was also reported that higher concentrations of IBAP or NAA (> 1.0 mg L^{-1}) inhibited root formation in mulberry (Vijayan *et al.*, 2014). Anis *et al.* (2003) reported that the highest rate of rooting in *M. alba* (80 %) was found on 1.0 MS medium plus 1.0 mg L^{-1} NAA.

Conclusions

The present study was the starting point to conserve *andaleh* (*M. macroura* Miq. var. *macroura*) *in vitro*. This is also the fundamental approach to develop the parental material for breeding program of *andaleh*. We also found that MS basal medium supplemented with low concentration of BAP (0.5-1.0 mg L^{-1}) would be used to induce shoot formation. On the other hand, the rate of callus formation was high (100%) in highest BAP concentration (2.0 mg L^{-1}).

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