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The effect of picloram concentrations and explants types on the induction of somatic embryo on North Aceh Cocoa genotype

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Abstract. The effect of picloram concentrations and three explants types on cacao somatic embryogenesis was studied. The objective of this work was to evaluate a procedure for somatic embryogenesis of cacao (*Theobroma cacao* L.) Aceh clones. Somatic embryogenesis was induced using floral tissue explants (petal, staminode, and anther) on North Aceh cacao genotype. No growth was observed on petal types of explants on low concentrations of picloram. Depending on the explants, the percentage of explants producing one or more embryos ranged from 3% to 75%, 5% to 80%, and 10% to 90% on media containing 3 mg/l, 4 mg/l, and 5 mg/l picloram respectively. Staminode explants produced 3 to 10 times more somatic embryos than petals, while anthers produced 2 to 5 times. Secondary embryos were induced from the floral explants of the North Aceh cacao genotype that exhibited efficient primary embryogenesis. Percentage embryogenesis generally increased with culture time.
Keywords: picloram, Cocoa, Somatic embryos, Genotype

Introduction

Aceh is one of the provinces in Sumatera that produce cocoa. Although the number of farmers and cocoa farms is increasing by year, but in terms of quality and quantity of cocoa production in Aceh is still very low. One of the reason is there are many cocoa plantations that use low quality seeds. In 2020, Aceh province is targeted to be one of the large cocoa producers in Sumatra with the highest seed quality. Therefore, in order to support the revitalization of the cocoa plantations in Aceh province, large amounts of seeds are needed. The superior of Aceh cocoa clones derived from cocoa plants adaptive in Aceh itself is the best way to use as seedlings. And consequently, plant breeders are required to assemble superior cocoa clones adaptive in Aceh in a short time.

Cocoa mostly propagated by generative way (75-90%) through F1 hybrids. Generative propagation through seed is relatively easy but the resulting plants are not uniform (Maximova *et al*., 2002). On the other hand, vegetative propagation is more difficult but the plants produced are more uniform. Cocoa plants from vegetative propagation (10-25%) are generally obtained through cuttings and grafting (Winarsih *et al*., 2003), and currently cannot fulfill the demand for cocoa seeds in bulk. Tissue culture techniques is one of the potential alternatives to solve these problems.

Tissue culture method is now widely used in plant breeding program to obtain genetic diversity or effort to find superior character efficiently without crossing process of plants. By using tissue culture methode, a new individual plant character different from the parental is possibel to obtain. The difference occur in the new plant indicate the occurrence of somaclonal variability. Li and Gray (2005) have said, factors that influence the occurrence of somaclonal variability during the tissue culture method include physiology factors, genetics and biochemistry. The effectiveness of somaclonal appear in tissue culture method depend on the eksplan used, method of inducing callus and regenerate it into plantlets (Mythili, *et al*., 1997). Factors that affecting callus induction and plant regeneration are choice of explant, genotype and media supplements used. This is also determined by the type of plant growth regulators such as auxin and cytokinin (Denchev & Conger, 1995). The aimed of this study is to determine the picloram concentrations that induce somatic embryos on explants derived from cocoa flowers adaptive in North Aceh. The study was conducted at the Tissue Culture Laboratory of the Faculty of Agriculture, Syiah Kuala University, Banda Aceh, from July 2012 to October 2012.

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Materials and Methods

Unopened immature buds about 3 to 5 mm long of field grown cocoa plant were collected in July 2012 from farmer plantation in North Aceh. The collection was done early in the morning. The explants preparation protocol used as mention in Young *et al*. (2003). Immature flower buds were collected in clean container containing cold water. The cold water then was decanted from the immature flowers buds and transferred all the flowers buds into the container with 5% (v/v) sodium hypochlorite solution and addition of 5 drops Tween 20 for 5 minutes. The flower buds were rinsed 5 times in sterile distiled cold water. The flower buds were collected and placed in 4^0C DKW basal sugar free media, the temperature was maintain (kept on ice) until the flower buds ready to dissect in the lab. Then immature flower buds were surfaced sterilized by immersion in 70% (v/v) ethanol for 20 seconds, followed by 15 min in 10 % (v/v) sodium hypochlorite solution and addition of 5 drops Tween 20, and then rinsed 5 times in sterile distiled water. Explants were prepared by briefltly blotting the immature flowers with sterile paper towels and separated them into petals, anthers and staminodes using a sterile scapel blade. Explants (petal, staminode, and anther) were first culture in Iinitiation Modium that contained DKW basal salts as described by Li *et al* (1998) supplemented with 250 mg glutamine L^{-1},100 mg myo-inositol L^{-1}, 2 mg 2,4-D L^{-1}, 1 mg BAP L^{-1}, 20 g glucose, and 7 g bacto agar. Petri dishes (100x15 mm) containing 20 mL of medium were used as culture vessels. All culture media were adjusted to a pH of 5.7 with 10 N KOH and autoclaved at 121 ^{0}C for 20 min. Cultures were maintained in the dark at 25^0C for 14 days.

After 14 days on initiation medium, explants were transferred onto secondary callus medium growth (SCG) and maintain for another 14 days under the culture conditions described above and sub cultured at 14 days interval. Various concentrations (1, 2, 3, 4, 5 mgL^{-1}) of picloram in combination with 1 mgL^{-1} of BAP in the secondary callus growth medium were evaluated for their ability to stimulate callus growth and somatic embryo production. Each treatment contained 5 explants per culture vessel, with seven replicate vessels.

The percentage of embryo-producing staminodes over the total number of cultured explants representing the frequency of embryogenesis, the average number of embryos produced from each responsive staminode were determined 6 weeks after culture initiation. Data were analyzed and graphs plotted using Excel software.

Results and Discussion

There was an enlargement of the staminode and anther explants cultured on the induction medium and followed by the development of compact callus over the explants. Frequently, globular callus clusters appeared over the surface of staminode, anther and petals. However callus on the petals fail to grow into futher step due to the contamination. Several effort have been tried out to overcome the contamination appear on the petals, but still didn't succesed yet. High contamination occured on the petal probably due to the high of microbe brought from the field. If the concentration of desinfectan used high during the explants sterilization, it will cause the explants tissues damage and even can kill the explant. While when using the low concentration of desinfectan will cause contamination persist.

The picloram concentration in the SCG medium significantly affected the percentage of explants that developed somatic embryos. Percentage callus induction for the explants type ranged from 80% on a picloram concentration of 3 mgL^{-1} to 0% on 1 mgL^{-1} picloram. The highest percentage explants live and the percentage of callus production found in staminode, follow by anther. In terms of number of somatic embryos, staminodes were the best with 5 somatic embryos formed per explant and 1 on anther. However, in terms of percentage callusing, staminodes cultured on 5 mgL^{-1} superior to those cultured on lower picloram concentrations. For example staminode cultured on picloram 5 mgL^{-1} had a callusing percentage of 60% , while the staminodes on a picloram concentration of 4, 3, 2, 1 mgL^{-1} had values of 45, 30, 25 and 0 % respectively.

An important step for the sucessful clonal propagation of plants through somatic is the availability of a culture procedure that would ensure somatic embryo production. Several previous reports showed the production of somatic embryos from various somatic tissues of cocoa but the efficiency of thes procedures was low (Sondahl *et al*., 1989, 1993). The reported

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rates of primary somatic embryo production from the culture of a large number of petal and nucellar explants were 4,3 and 2,0%, respectively (Sondahl *et al*., 1993).

In our previous studies on somatic embryogenesis in cocoa, MS medium, Murashige and Skoog (1962) was used as the main source of inorganic nutrients suplemented with BA and picloram. The results showed that none of somatic embryos formed on the three types of explants (staminode, anther and petal) used, the tissues were senescence and necrosis. This kind of results also found on the work done by the cocoa research institute when using MS medium supplemented with NAA and kintin. Which often resluted in reduced growth, rapid senescence and eventually tissue necrosis (data not shown). DKW medium for the *in vitro* propagation of woody perennial species, provide high concentration of Calcium, Sulphur and Magnesium than the MS medium. These elements are essential for cell differentiation and somatic embryogenesis (Pedroso *et al*., 1996).

Based on our experience on the induction of somatic embryos in peanut, the use of picloram were able to induce the formation of somatic embryos. Changes resulting from the treatment of tissue culture techniques can lead to an improvement or decline in the character, and it indicates the occurrence of somaclonal variable (Zuyasna *et al*. 2005). The existence of somaclonal variability that occurs in the tissue culture techniques can be used to generate new desired clones of plant. Factors that influence the emergence of somaclonal variation among the cells or tissue cultured plants *in vitro* are: length of tissue culture *in vitro*, explants source used, the type of regeneration, donor plant genotype, concentration and type of growth regulator used or selective media conditions used *in vitro* (Amberger *et al*., 1992, Skirvin *et al*. 1993).

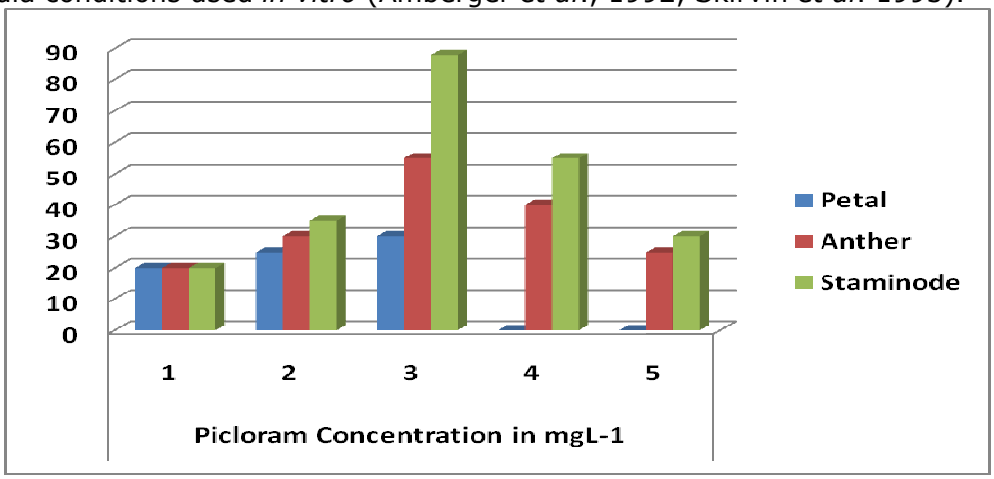

Fig.1. Effect of different concentration of picloram in the SCG medium on the percentage mean explant responsive cultured for 1 month at 20^0C.

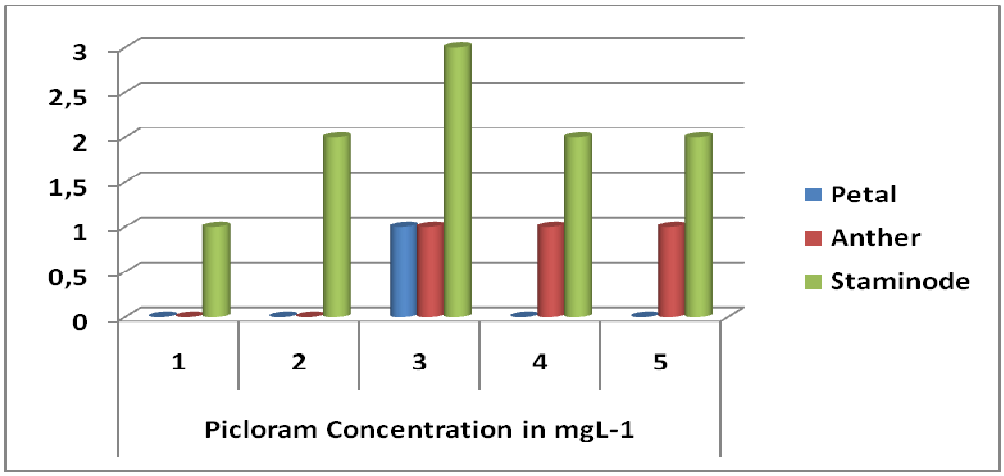

Fig.2. Effect of different concentration of picloram in the SCG medium on the mean number of somatic embryos from petal, anther, and staminode cultured for 1 month at 20^0C.

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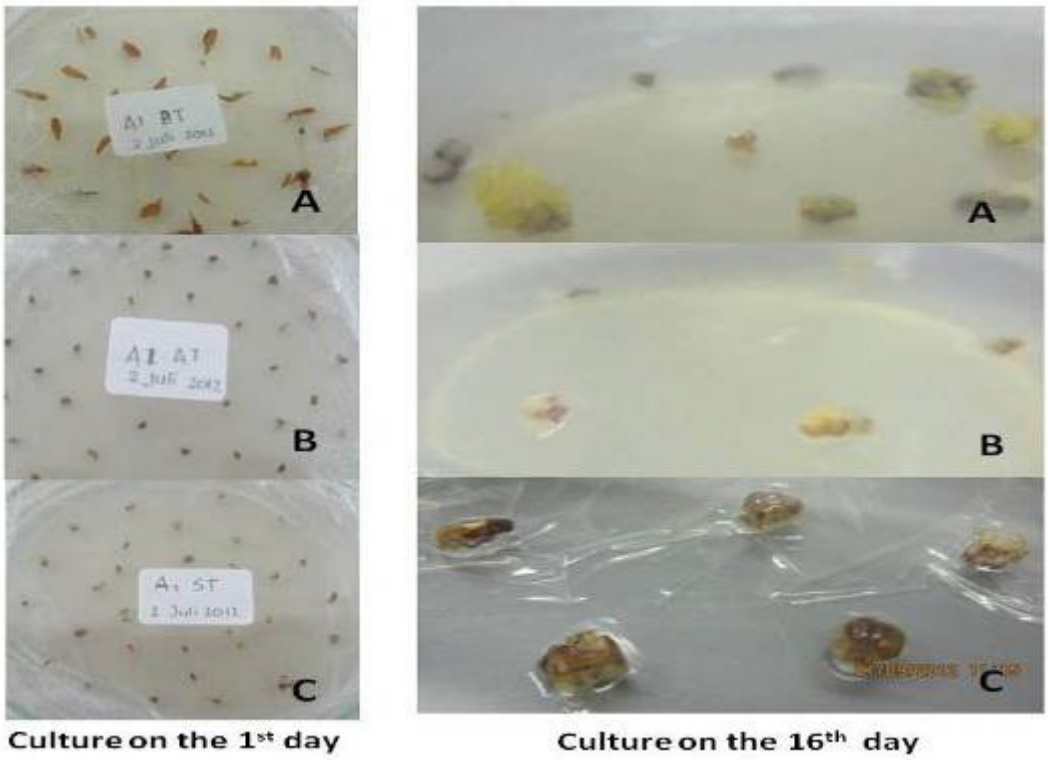

Fig. 3. Different explants exposed on the DKW medium contained 3 mgL^{-1} picloram. A= petal; B = anthers; C = Staminode

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