IN VITRO PROPAGATION OF SUGARCANE (*Saccharum officinarum* L.) OF CM2012 TAKALAR VARIETY

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ABSTRAK

Kultur jaringan menjadi pilihan untuk produksi bibit tanaman secara massal dan bebas patogen. Untuk mengetahui komposisi kombinasi zat pengatur tumbuh (ZPT) utama yang menghasilkan propagula tebu secara efektif, dilakukan kultur in vitro tanaman tebu menggunakan gulungan daun dan meristem apikal sebagai eksplan. Kedua sumber eksplan tersebut dikulturkan pada medium MS yang dilengkapi ZPT Auksin: 1-napthaleneacetic acid (NAA) + Kinetin (untuk gulungan daun) dan 6-benzyladenine (BA) atau Kinetin (untuk meristem apikal) pada berbagai kombinasi dan konsentrasi yang berbeda, sehingga diketahui kombinasi dan konsentrasi yang paling efektif menghasilkan plantula tebu. Hasil percobaan menunjukkan bahwa perlakuan pemberian hormon tumbuh berpengaruh sangat nyata terhadap pertumbuhan eksplan tebu. Di antara perlakuan tersebut, penambahan Kinetin 1 $mg.L^{-1}$ yang dikombinasikan dengan NAA 0.5 $mg.L^{-1}$ menghasilkan jumlah tunas, jumlah daun serta panjang akar yang terbaik untuk eksplan gulungan daun. Untuk eksplan meristem apikal, penambahan Kinetin 2 $mg.L^{-1}$ menghasilkan plantula lebih banyak dibandingkan dengan yang dihasilkan melalui eksplan gulungan daun, namun jumlah akar lebih sedikit sehingga diperlukan subkultur ke media perakaran. Eksplan meristem apikal yang ditumbuhkan pada medium MS dengan penambahan ZPT BA gagal tumbuh.

Kata kunci: Tebu, kultur jaringan, gulungan daun, meristem apikal

ABSTRACT

In vitro propagation has become a choice for large-scale production and setting up a large pathogen free delivery system in sugarcane. This study was conducted to find an effective regeneration method for mass propagation of sugarcane of a commercial variety of Takalar CM 2012. Leaf sheath and apical meristem were used as explants and cultured on MS medium supplemented with Kinetin alone for apical shoot and on combination of either Kinetin + NAA or BA + NAA at various combination of concentration. The research was design in a random completed design which comprised of five replicates and every replicate consisted of six observation material units. The multiple shoot regeneration at various frequencies was observed by using different concentration and combination of growth regulators. The best response in terms of multiple shoot induction was observed on apical meristem explants cultured on MS medium with Kinetin 2.0 mg/l, but produced fewer roots as compared to other treatments. A combination of Kinetin 2.0 mg/l and NAA 0.5 mg/l gave the highest result on the number of multiple shoot and roots of Leaf sheath cultures. No growth were observed when the leaf sheath explants were cultured on MS medium containing BA 1.5 mg/l + NAA 0.5 mg/l and BA 2 mg/l + NAA 0.5 mg/l. Apical shoot explants also failed to grow on MS medium supplemented with BA 2 mg/l. Hence, the growth of sugarcane variety of Takalar CM 2012 on in vitro culture was influenced by cytokinin in combination with auxin at low concentration.

Key words: Sugarcane, in vitro propagation, leaf sheath

INTRODUCTION

Sugarcane is an important industrial crop and cultivated as a commercial crop in tropical countries as the main source of raw material for the sugar production. The plant also capable of providing renewable energy sources such as ethanol, biogas byproducts and fertilizers.

As a tropical country, Indonesia has been producer of sugarcane since the seventeenth, however the national sugar production is lower than the domestic needs and it makes Indonesia as a sugar importer country. To meet the growing demand of sugarcane products, the government is paving the way to increase the sugar production through revitalization of sugar industry and enlargement of sugarcane plantations. In 2015, the government has given license for expansion of 215,000 ha plantation in several provinces with the objective to achieve self-sufficiency in sugar production (Dep. of Energy Technology 2015).

In sugarcane, conventional propagation is through sets, which is slow, usually one to ten in a period of one year. Moreover, pathogens keep on accumulating generation after generation which reduces the yield and quality of sugarcane (Biradar 2009). Therefore, the use of in vitro

propagation for large-scale production and setting up a large pathogen free delivery system in sugarcane is needed. Micropropagation of sugarcane has done successfully using sheath leaf and apical meristem/shoot tip. Initial growth, multiplication shoots and development of vigorous plantlets occurred when the two different explants incubated on Murashige and Skoog (MS) medium under the influence of plant growth hormones particularly auksin dan sitokinin (Roy & Kabir 2007; Ali et al. 2008; Wagih et al. 2009; Tarique et al. 2010). Despite the advantages of *in vitro* technique, phenotypic instability or somaclonal variation as a result of the occurrence of genetic variants derived from in vitro procedures is supposed to arise. Factors such as explant source, number of subculture, and growth hormones are identified capable to induce *in vitro* variability.

In terms of explant source, explant from leaf have highest potential for callus formation, proliferation and planlets generated from these undiferentiated cell mass are also high. However, in callus phase there are the maximum chances of mutation and somaclonal variation, the plants which are regenerated from the callus are not true-to-true types for their chromosomal aberration and many new characters found from the plants (Karim et al. 2002; Sood, 2006; Biradar 2009). Option available currently to get true to type plants is to secure enhanced release of axillary buds through apical meristem or shoot tip culture. Although the rate of multiplication is low in shoot tip culture but variation is nil or very less, hence micropropagation coupled with apical meristem culture technique ensures the production of whole plants that are generally identical (Hoy, 2003; Biradar, 2009).

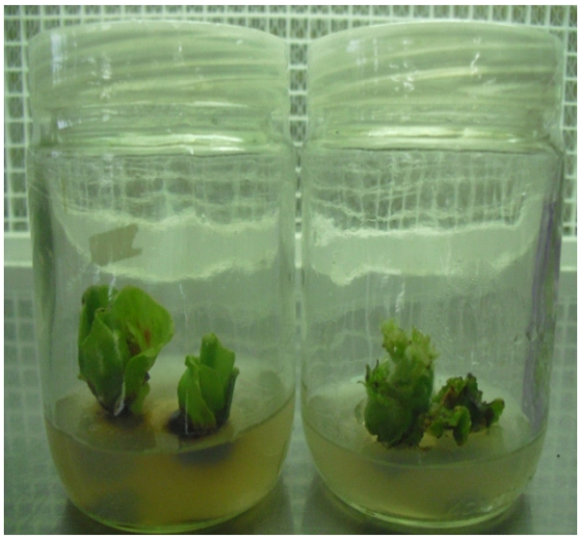
The occurance of somaclonal variation on leaf or callus cultures has been described (Hoy et al. 2003; Khan et al. 2008; Khan et al. 2008; Sobhakumary, 2012), however other factors such as subculture and growth hormones may also influence the in vitro variability. Therefore, the present experiment was conducted to regenerate plants by in vitro technique through apical meristem and folded leaf cultures in sugarcane for further evaluation on the occurance of somaclobal variation at a field experiment. This communication accordingly deals with the studies of direct shoot regeneration from apical meristem and leaf sheath, multiple shoots and root induction

MATERIALS AND METHODS

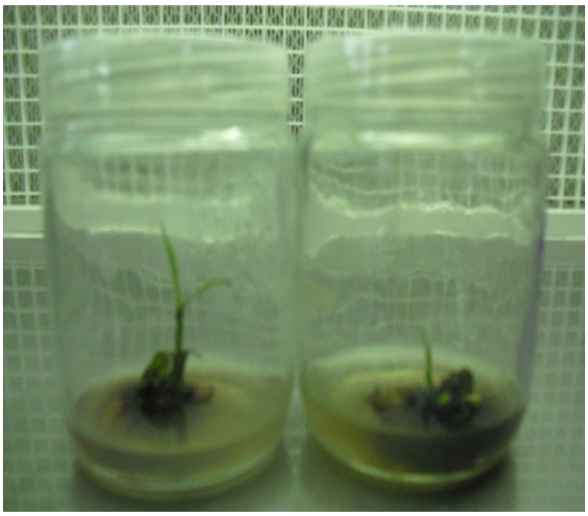
Shoot containing shoot tips and folded leaves of a mature plants of sugarcane var. CM2012 were collected from the field grown of Takalar's Sugarcane Estate (South Sulawesi, Indonesia). Shoots were washed throughly under running tap and subsequent sterlization was carried out in the laminar air flow cabinet under aseptic condition. The spindle explant was immersed on to 70 % alcohol then subjected to flame and the leaf sheath around the spindle were removed aseptically with the help of a pair of forceps and a scalpel blade. This procudere step was done several times until the segment of tightly rolled internal leaves was found. For leaf-roll callus culture, the inner rolled leaves was excised from the stalk apex and cut into small pieces (1 - 1.5 cm in length and 1 cm in dimater) and inoculated on agar solidified MS supplemented with combination of either Kinetin + NAA or BA + NAA at various concentration. Upon establishment, they were transferred and maintained on the same medium at 30 days interval. The subcultures were done twice to promote multiple shoots and healthy plantlets formation. For meristem culture, the apical meristem was excised, placed on agar solidified MS supplemented with Kinetin. The initiated shoots were also subcultured twice on the same fresh medium for multiplication and elongation. The elongated shoots were subsequently rooted on MS fortified with NAA (2.0 mg/l). MS medium used contained 3% sukrosa, 8 g/l of agar, media pH adjusted to 5.7 prior to autoclaving. Cultures were incubated at 25±2°C with 16 hours of light provided by cool white fluorescent tubes and data were recorded 30 days after inoculation.

RESULTS AND DISCUSSION

Early growth of the cultures was observed after seven days of inoculation. Enlargement, swelling, browning and unfolded leaves were appeared at all cultures investigated. Cut ends of the leaf sheat explants became swollen and began to produce tiny shoots after 20 days of culture, later, adventitious shoot proliferation was observed from cut ends and mid ribs of leaves (Picture 1). In apical meristem cultures, auxiliary shoot grew from nodes which leaf base adjacent to the stem (Picture 2). Shoot proliferation from the two different explants differed accoding to the growth regulators and combination concentration of cytokinin and auxin used (Table 1). Among the combination of growth hormones used, combination concentration of KN 1 mg/l + NAA 0.5 mg/l was found to be the best for shoot generation, in which 90% of folded leaf explants regenerated shoots. The average number of shoot produced per explant was 51.91 and the number of leaves as well as the roots length were also the highest observed in this medium. On the other hand, the growth of folded leaf explants in the medium supplemented with BA + NAA were less optimum, in which more explants failed to grow (Table 1).



Picture 1. The early growth of folded leaf explants



Picture 2. Shoots initiated from apical meristem cultures of sugarcane variety Cm2012 In apical meristem cultures, it was obvious that kinetin was more effective in regeneration of sugarcane than BA. 90% of the explants induced shoots in the medium fortified with 2 mg/l kinetin and the number of shoots produced was higher as compared to that resulted in folded leaf explants. This result is in contrast to the result of a study by Karim et al. (2002) where BA was superior over kinetin for producing shoots. These observations suggested that type and concentration of cytokinin required for shoot regeneration and establishment varies with genotypes. Another kind of cytokinin (Benzylamino purine or BAP) reported by Biradar et al. (2009) was effective in inducing and multiply shoots, the average rate of multiplication (79.64) observed at 2 mg/l BAP. However shoots were weak, tiny and non separable making it difficult to manage. Therefore, to produce well grown, easily separable shoots and healthy plantlets, the use of lower BAP concentration was recommended

Table 1. Effect of the cytokinin and the auxin at different combination and concentration in MS medium on shoot regeneration from the folded leaves and apical meristem tissue of sugarcane variety Cm20012

Growth hormones (mg/l)	% of explant produced shoots	Time required for shoot initiated (days)	Number of shoots per explants	Number of leaves	Root lenght (cm)
Leaf sheath					
KN1NAA0.5	90	11.89dc	51.91b	105.83ab	12.12a
KN1.5NAA0.5	75	17.70b	18.53e	56.11cd	7.56b
KN2NAA0.5	75	12.39c	24.08c	49.67d	6.13bc
BA1NAA0.5	80	9.27a	22.67cd	76.89c	4.41e
BA1.5NAA0.5	-	-	-	-	-
BA2NAA0.5	-	-	-	-	-
Apical Meristem					
KN2	90	22.01e	61.66a	136.79a	4.67d
BA2	-	-	-	-	-

Means followed by a different letter in each column are significantly different at DMRT (*P= 0.05)*.

Various concentration of cytokinin (BA and Kinetin) and auxins (NAA) were used in different concentration and combinations for shoot regeneration from leaf sheath explants. As can be seen from the Table 1 that shoot formation was highly influenced by concentrations and type of the growth regulators used. Among different concentrations and combinations for shoot multiplication, best performance was showed on MS medium supplemented with Kinetin 1.0 mg/l + NAA 0.5mg/l. The second best performance was found on MS medium supplemented with Kinetin 2.0 mg/l + NAA 0.5 mg/l in which average number of usable shoots was 24.08 with number of leaves 49.67. This results revelead that Kinetin was effective in producing multiple shoots at low level. The results of this study is consisten with previous reports by Karim et al. (2002) and Tarique et al. (2010) where, combination concentration of cytokinin 1.5 mg/l and auxin 0.5 mg/l were found to the best for shoot regeneration and multiplication. However, all these studies concluded that combinations of high level of cytokinin and a low level of auxin were essential for differentiation of adventitious shoot in sugarcane.

It was also evident from Table 1 that all combination of Kinetin and NAA were suitable for rooting. Profuse rooting was observed in the cultures after 30 days following the first subculture. The growth of roots in the folded leaves culture was likely influenced by NAA that added to the medium, as reported by previous studies that NAA is most suitable auxin for rooting particularly in sugarcane (Roy & Kabir, 2007; Biradar et al., 2009; Behera & sahoo, 2009; Tarique et al., 2010). After 8–9 weeks, vigorous rooting was observed, after full establishment of roots (7.56 to 12.12 cm long).

At the meristem apical cultures, no roots were observed in the medium containing cytokinin, therefore, the elongated shoots were transfered to medium suplemented with NAA 2 mg/l.

Picture 3. Rooted plantlets regenerated from meristem apical explants.

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