Comparison of the Reduction Effect of Sucrose and Table Sugar Concentration on Growth Characteristics of Red Ginger (*Zingiber officinale* Rocs.) Cultured in Liquid Medium

Betalini Widhi Hapsari*, Tri Muji Ermayanti, Deritha E. Rantau, and Rudiyanto

Research Center for Biotechnology, Indonesian Institute of Sciences (LIPI), Indonesia

Abstract

The aim of this research was to compare the reduction effect of sucrose or table sugar concentration on growth characteristics of red ginger cultured in MS liquid medium. Shoots of red ginger was cultured on MS liquid medium without addition of plant growth regulators, supplemented with 5, 10, and 20 g/l of sucrose or table sugar for 8 weeks. Resulted plantlets were acclimatized in a greenhouse to investigate their growth and survival rate. Numbers of stomata, chlorophyll concentration as well as cross section of leaves from plantlets grown *in vitro* were compared to those of transplants grown in the greenhouse. The results showed that the use of table sugar at concentration of 20 g/l gave the best growth of red ginger. Meanwhile, the reduction of table sugar from 20 to 10 g/l reduced growth and survival rate of *in vitro* shoots as well as that of transplants in the greenhouse. Only few shoots formed roots when they were grown on the medium containing 5 g/l of table sugar, and transplants failed to grow in the greenhouse. It found that the chlorophyll content of *in vitro* plantlets was lower than those of transplants grown in the glasshouse. However, the number of stomata of the *in vitro* plantlets was higher than that of transplants grown in the glasshouse. There was no anatomical abnormalities found on the cross section of leaves between *in vitro* plantlets and transplants grown in the greenhouse. The replacement of sucrose with table sugar may reduce the production cost of plantlets.

Keywords: red ginger (*Zingiber officinale* Rocs.), reduction of sugar, survival rate, chlorophyll, stomata.

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*Corresponding author
Jalan Raya Bogor Km 46, Cibinong 16911, Indonesia
Tel. +62-21-8754587, Fax. +62-21-8754588
E-mail. betalini.widhi.hapsari@lipi.go.id; betalini_widhi@yahoo.com

Introduction

Ginger (*Zingiber officinale* Rosc.), originated from Central and South Asia, is an important medicinal crop in the tropical and subtropical Asia regions. The major ginger producing nations are China, India, Brazil, Jamaica, and Nigeria (Babu *et al.,* 1992). Ginger's pungent aromatic rhizome is consumed all over the world as a spice, culinary herb, condiment, home remedy, and medicinal agent, but mostly used as herbal plants and medicines due to the unique character of ginger essential oil and ginger oleoresin. Fragrant aroma of ginger is caused by essential oils, while oleoresin causes a spicy flavor. The content of volatile oil in dry ginger is about 1-3%. The main components of essential oil causing the fragrant smell of ginger are zingiberol and zingiberen. Ginger oleoresin contains a lot of components to build a spicy flavor that does not evaporate. The component in ginger oleoresin consists of gingerol, zingiberen, shagaol, essential oils, and resins, while the ginger spicy flavored components is zingiberol. In addition to these contents, ginger rhizome also contains starch and organic acids (Sutarto *et al.,* 2003). Those compounds make ginger rhizome plants have high economic value.

Increasingly the wide use of ginger plants as food, beverages, and cosmetics or in the form of essential oil, cause the demand for ginger, especially red ginger continuously increases. Red Ginger is different from other types of ginger due to higher volatile oil and oleoresin content (Tim Lentera, 2002). Ginger demand increases in line with the increasing development of traditional medicine industry and other industrial ginger-based raw material. Based on data from National Statistical Board (BPS) of Indonesia in 2009, ginger production from 2005 to 2007 was 125.857 tons, 177.138

tons, and 178.503 tons, respectively (BPS, 2009).

Ginger is generally reproduced vegetatively through its root rhizomes. Tissue culture method is needed to produce high quality of transplants. Most reports on the micropropagation of ginger were usually report about modification of plant growth regulators added to the culture medium for the improvement of plantlet growth. An experiment on the manipulation of *in vitro* condition and the reduction of sugar on a liquid medium have enhanced growth and development of red ginger (Hapsari *et al.*, 2009; Ermayanti *et al.,* 2010). Furthermore, from previous studies showed that the *in vitro* propagation of the red ginger could be performed on liquid MS medium without addition of growth regulators (Hapsari *et al.*, 2009; Ermayanti *et al.*, 2010). In order to minimize the production cost of red ginger tissue culture, the aim of this research was to compare the reduction effect of sucrose or table sugar concentration on growth characteristics of red ginger cultured in MS liquid medium.

Materials and Methods

Shoot initiation and multiplication. Red ginger rhizome used in this study was obtained from Bogor area. Initiation of shoots from rhizome buds was conducted on MS medium with the addition of 8 g/l agar (Murashige & Skoog, 1962). Buds were sterilized by sodium hypochlorite and fungicides then they were planted on MS solid medium and then incubated in culture room at 26-27°C, with continuous photoperiod using fluorescent light. The intensity of light was 1,000-1,300 lux. Shoot multiplication was done by transferring *in vitro* shoots to liquid MS medium without addition of plant growth regulators. Shoot cultures on solid media were placed on the culture shelf while cultures on liquid media were kept on a shaker with 90 rpm. Both cultures were incubated in the culture room with the same condition with shoot initiation.

Treatment with reduction of sucrose and table sugar concentration. MS liquid medium containing sucrose 20 g/l was used as a control treatment. Reduction of sucrose concentration to 10 and 5 g/l was used as treatments. The growth of red ginger was also compared with the used of table sugar to replace sucrose at concentration of 20, 10, and 5 g/l. *In vitro* stems with no leaves isolated from the multiplication medium were used as explants. Explants were cultured on MS liquid medium with no addition of plant growth regulators with 6 replicates for each treatment. Observation on the growth was conducted after 8 weeks of culture by recording the number of multiple shoots, height of plant and number of roots.

Chorophyll analysis. Chlorophyll observation was carried out following the method of Meeks (1974). About 0.1 g fresh leaves were taken from plants growing in the greenhouse as well as from *in vitro* shoots. Leaves were then cut into small pieces, then extracted with the addition of 10 ml of ethanol 95%. Extracts were then put in the tube and rotated with a vortex for 20 min. The liquid was separated from the sediments and the absorbance was measured using spectrophotometer at wavelengths of 663 and 645 nm. Calculation of chlorophyll-a (mg/g of leaf weight) was $(12.7 \times A_{663}) - (2.69 \times A_{645} \times 10^{-1})$, while chlorophyll-b was $(9.22 \times A_{645}) - (4.68 \times A_{663} \times 10^{-1})$. Total chlorophyll was $(8{:}02 \times A_{663}) + (2.20 \times A_{645} \times 10^{-1})$.

Calculation of stomata number. Number of stomata was recorded on leaf epidermal strips at upper and lower epidermis using a light microscope with a magnification of 200 times (Olympus CX 41 series).

Acclimatization. Acclimatization was carried out by transferring plantlets in the polybag containing a mixture of soil, compost and sand with ration 1: 1: 1, plantlet was planted on polybag. Plantlets were covered by plastic until they formed a new leaf. Plantlets were placed in the shade area before transferring to the greenhouse. One month after planting, the plastic cover was removed and the plantlets were transferred to the greenhouse. Observations were done by recording the number of survival plantlets every two weeks up to 8 weeks. Number of shoots, height of plants and the number of roots were also recorded.

Statistical analysis. All data presented in this

research were analyzed by Anova at 5% level, and advanced test used Duncan's Multiple Range Test (DMRT) at 5% level.

Results and Discussion

Reduction of sucrose concentration affected the growth of red ginger shoots on liquid MS medium with no addition of plant growth regulators. Reduction from 20 g/l to 10 g/l of sucrose reduced the number of shoots, height of plantlets as well as the number of roots. Lower concentration of sucrose (5 g/l) gave poor growth and none of the plantlets survived in the greenhouse. Meanwhile, the application of table sugar at concentration of 20 g/l gave a better growth of red ginger compared to the same concentration of sucrose. However, reduction of table sugar concentration to 10 and 5 g/l reduced growth significantly (Table 1).

Table 1. Growth characteristic and survival rate of red ginger (*Zingiber officinale* Rocs.) cultured in MS liquid medium without plant growth regulators, supplemented with different concentrations of sucrose or table sugar at week-8 after acclimatization

Concentration (g/l)	Numbers of shoots	Height of plants (cm)	Number of roots	Survival rate (%)
Sucrose				
20	5.50 b	6.75	6.17 bc	50.0
10	4.17 ab	5.00	2.83 ab	40.0
5	2.83 a	3.75	0.33 a	0
Table sugar				
20	5.67 b	8.00	8.33 c	40.0
10	2.83 a	4.63	1.67 a	25.0
5	2.83 a	4.25	0.83 a	0

Note: Value followed by the same letter on the same column is not sugnificantly different at P. values of 0.05 according to Duncan's Multiple Range test

Growth characteristic of plantlets at week-8 after acclimatization is represented in Figure 1. Only plantlets originated from MS liquid medium containing sucrose or table sugar at concentrations of 20 and 10 g/l survived in the greenhouse. Meanwhile, all plantlets grown on the MS liquid medium containing either sucrose or table sugar at concentration of 5 g/l did not survive in the greenhouse (Table 1). On the medium containing either sucrose or table sugar at concentrations of 20 and 10 g/l, number of leaves started to increased at week-2 after acclimatization (Figure 1A). At the beginning of acclimatization, plantlets originated from the MS liquid medium containing 20 g/l of sucrose had 2.75 leaves, then increased slowly at week-2 and week-4, and remained stable with having 8 leaves/plantlets at week-6 and week-8. Plantlets grown on MS liquid medium containing 10 g/l of sucrose, formed more new leaves on week-2 and week-4 compared with plantlets cultured on medium containing sucrose of 20 g/l, but, then decreased slowly on week-6 and week-8. This could be due to the plantlets movement from the shade area to the greenhouse and could be related to the removal of plastic cover. This indicated that the change of environmental condition such as temperature and moisture may affect formation of leaves. Similar growth was also observed for plantlets grown on the culture medium containing table sugar at concentration of 20 and 10 g/l. At week-8 after removal of plastic cover, the number of leaves was also reduced (Figure 1A).

Plantlets from the medium containing 10 g/l of sucrose started to form a new shoot at week-4 (Figure 1B). All plantlets from the medium containing either 20 g/l of sucrose or table sugar (10 and 20 g/l) started to form new shoots at week-8. The number of shoots was varied depending on the concentration of the sucrose or table sugar. The higher number of buds was achieved by plantlets originated from the culture medium containing 20 g/l of table sugar. This result indicated that the addition of sucrose at concentration of 20 g/l could be replaced with sugar at the same concentration. Since table sugar was much cheaper than sucrose, thus the large scale production of red ginger tissue culture, the production cost will be more economically. The observation was continued after 8 weeks

in the greenhouse to confirm the survival rate of the plantlet. The addition of fertilizer for acclimatization is also required to increase the survival rate of these plantlets. The use of fertilizer may be recommended to increase the plant growth in the greenhouse.

Height of plantlets increased during the acclimatization process up to week-6, however, plantlets originated from the medium containing 20 g/l of either sucrose or table sugar reduced afterwards (Figure 1C). This could be due to the change of the environmental condition from the shade area to the greenhouse and also because of the removal of plastic cover. Plantlets originated grown on the medium containing 10 g/l of either sucrose or table sugar grew slowly at the beginning, increased the height from week-4 up to week-8. However, the number of survival rate of plantlets grown on this medium was lower than the plantlets grown on the medium containing 20 g/l of sucrose or sugar.

Number of stomata and the chlorophyll content of plantlets grown *in vitro* and *ex vitro* are shown at Table 2. Reduction of sucrose or table sugar concentration resulted in lower number of stomata as well as the chlorophyll content. This may also affected the survival rate of the plantlets. All plantlets originated from the medium containing 5 g/l of sucrose or table sugar did not survive in the greenhouse. However, the growth and the number of roots may be more important than the length of roots for the red ginger to survive in the greenhouse, since the number of stomata and chlorophyll content were only slightly different when it was compared to the plantlets originated from the medium containing 10 g/l of sugar (Table 2). Both *in vitro* and *ex vitro* plantlets had normal leaves, shown from the cross section of their leaves (Figure 2). No abnormalities of the structure of the leaf tissue were found both from *in vitro* and *ex vitro* plantlets.

Value followed by the same letter on the same column is not sugnificantly different at P.values of 0.05 according to Duncan's Multiple Range test

Plantlets grown on MS medium containing 20 and 10 g/l of both sucrose and sugar showed normal and healthy growth, having green leaves and good performance (Figures 3A, B, C, and D). They survived in the greenhouse. Shoots grown on the MS liquid medium containing 5 g/l sucrose or table sugar had poor growth, did not formed roots (Figures 3E and F), so they could not survive in the greenhouse.

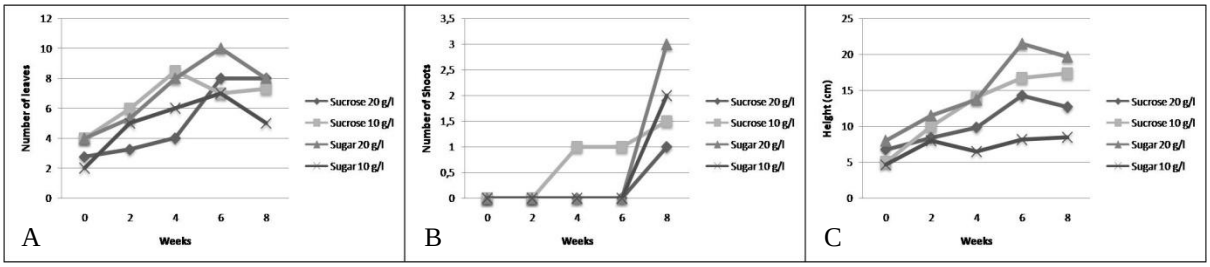

Figure 1. Number of leaves (A), number of shoots (B), and (C) height of shoots of red ginger (*Zingiber officinale* Rocs.) grown in the greenhouse

Table 2. Number of stomata and chlorophyll concentration of red ginger (*Zingiber officinale* Rocs.)

Concentration (g/l)	No. of stomata (upper epidermis)		No. of stomata (lower epidermis)		Chlorophyll-a		Chlorophyll-b		Total Chlorophyll	
	in-vitro plants	*Ex vitro* plants	*In vitro* plants	*Ex vitro* plants	*In vitro* plants	*Ex vitro* plants	*In vitro* plants	*Ex vitro* plants	*In vitro* plants	*Ex vitro* plants
Sucrose										
20	4.67 b	5.50	34.73 b	33.57 b	0.483 b	0.660 a	0.360	0.415 a	0.843	1.075 a
10	4.83 b	7.17	34.53 b	17.63 a	0.488 b	0.650 a	0.386	0.505 ab	0.873	1.155 a
5	1.97 a	-	26.13 a	-	0.291 a	-	0.266	-	0.556	-
Table sugar										
20	3.93 ab	4.43	37.97 b	26.53 ab	0.496 b	1.180 c	0.382	0.890 c	0.880	2.070 b
10	1.50 a	4.80	23.60 a	22.03 ab	0.414 ab	0.970 b	0.356	0.700 bc	0.769	1.670 b
5	1.53 a	-	23.47 a	-	0.393 ab	-	0.325	-	0.718	-

Note: -: Plants did not grow in greenhouse

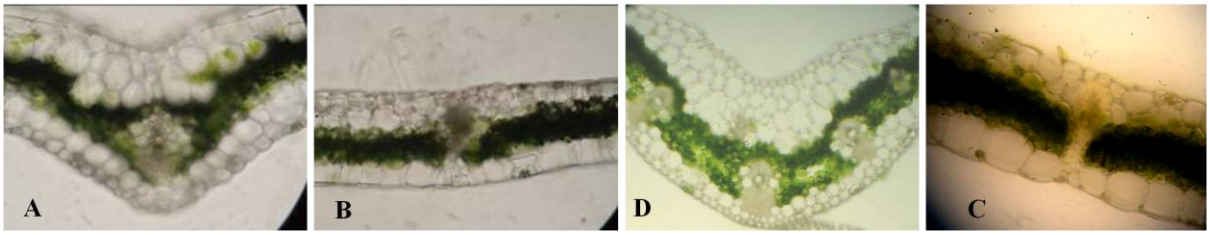

Figure 2. Cross section of red ginger (*Zingiber officinale* Rocs.) leaves grown *in vitro* (A and B) and *ex vitro* (C and D)

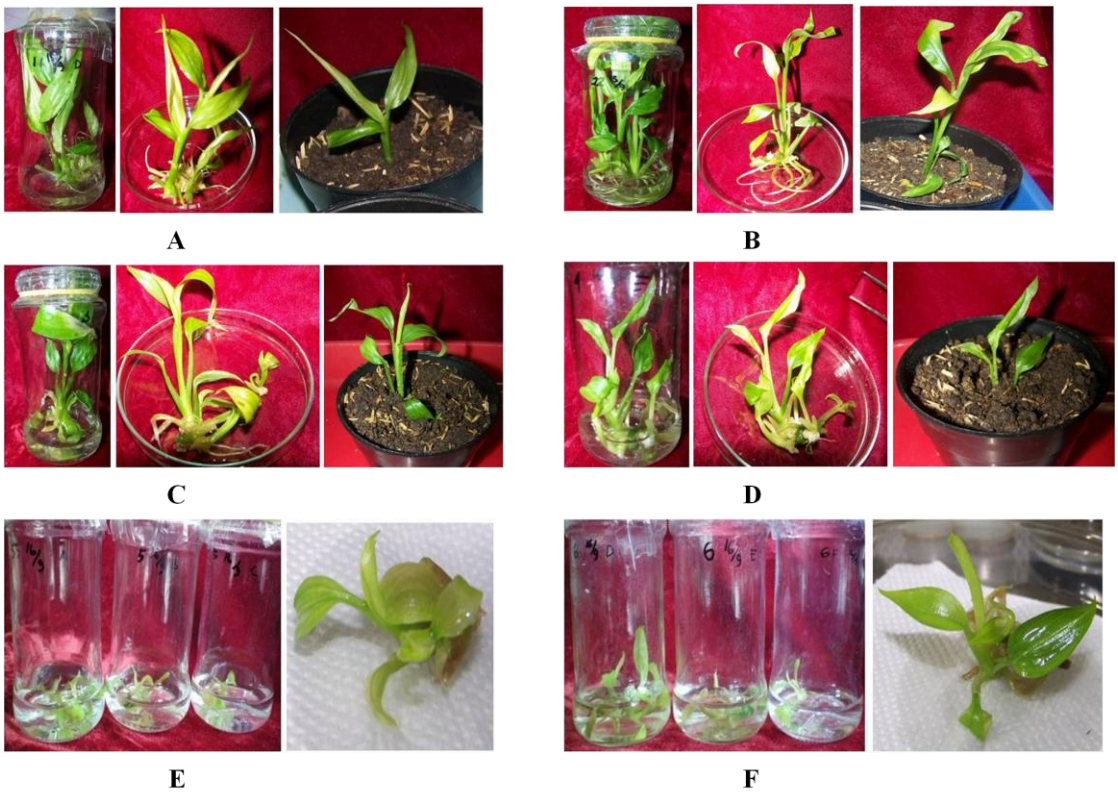

Figure 3. Red ginger shoots grown on MS liquid medium containing different concentration of sucrose or table sugar, and acclimatization of plantlets. A. Shoot on MS medium containing 20 g/l of sucrose; B. Shoots on MS medium containing 20 g/l of sugar; C. Shoot on MS medium containing 10 g/l of sucrose; D. Shoot on MS medium containing 10 g/l of table sugar; E. Shoot on MS medium containing 5 g/l of sucrose; F. Shoot on MS medium containing 5 g/l of table sugar

Replacement of sucrose with table sugar in liquid medium, elimination of solidifying agent, as well as plant growth regulators are important for large scale multiplication of red ginger tissue culture, because it would make the cost production is more economically. Using King Yai ginger cultivar from Thailand, the best multiplication medium was MS solid medium containing 5 mg/l of BA in combination with 0.5 mg/l NAA (Pandey *et al.*, 1997). An Indian ginger cultivar needed addition of 2.5 mg/l of BA in combination with 0.5 mg/l kinetin to reach the best multiplication rate of shoots (Khatun *et al.*, 2003). The replacement of sucroce at 20 g/l with table sugar at the same concentration gave better growth of the red gingger, so that the production cost can be reduced.

Conclusions

MS medium containing 20 g/l of table sugar was the best medium for the propagation of the red ginger. In this medium, the number of shoots, roots, chlorophyll content as well as the number of stomata was higher when it compared with the addition of sucrose with the same concentration. The application of table

sugar concentration at 10 g/l showing normal growth and development, however the reduction of table sugar concentration to 5 g/l resulted in poor growth and plantlets failed to grow in the greenhouse. Reduction od sucrose to the concentration of 10 or 5 g/l reduced growth of the in vitro red ginger. The addition of 20 g/l of table sugar could reduce the production cost as well as give better growth that the additon with 20 g/l of sucrose.

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