ORIGINAL ARTICLE

Effects of carbon sources and strength of MS medium on germination of somatic embryos of oil palm (*Elaeis quineensis* Jacq.)

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Abstract

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Various sources of carbon and strengths of Murashige and Skoog (MS) medium were investigated for their effect on oil palm somatic embryo germination or induction of shoots and roots from haustorium-staged embryos. It was found that the highest percentage of shoot formation was obtained in 0.2 M sorbitol containing basal MS medium. A high concentration of alcohol sugar and reduced strength of MS medium enhanced root formation. A high concentration of sucrose or sorbitol also promoted root formation. Percentage of root formation was the highest (31.25%) when excised shoots were cultured on root induction medium supplemented with 0.2 M sucrose.

Key words : oil palm, osmoticum, somatic embryo, shoot induction, root induction

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บทคัดย่อ

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ผลของแหล่งคาร์บอนและองค์ประกอบของธาตุอาหารสูตร MS ต่อการงอกของโซมาติกเอ็มบริโอปาล์มน้ำมัน (*Elaeis quineensis* Jacq.)

ว. สงขลานครินทร์ วทท. ธ.ค. 2548 27(ฉบับพิเศษ 3) : 629-635

ศึกษาชนิดของน้ำตาลซึ่งเป็นแหล่งของคาร์บอนที่แตกต่างกัน ร่วมกับการลดองค์ประกอบของธาตุอาหารสูตร MS เพื่อชักนำการงอก หรือชักนำการสร้างยอด และรากของโซมาติกเอ็มบริโอของปาล์มน้ำมันในระยะสร้างจาว พบว่าการเติมแมนนิทอลเข้มข้น 0.2 โมลาร์ ในอาหารสูตร MS ปกติ ส่งเสริมการงอกของโซมาติกเอ็มบริโอสูงสุด 40% ซูโครส แมนนิทอล (น้ำตาลแอลกอฮอล์) ความเข้มข้นสูง และการลดองค์ประกอบของธาตุอาหารส่งเสริมการสร้างราก เมื่อนำยอดของปาล์มน้ำมันมาเพาะเลี้ยงบนอาหารสูตร MS เติมน้ำตาลซูโครสเข้มข้น 0.2 โมลาร์ พบว่าส่งเสริมการสร้างรากสูงสุด 31.25%

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Oil palm (*Elaeis quineensis* Jacq) is a very important commercial crop in southern Thailand. Propagation through tissue culture is widely used. A working team from Malaysia recently found that cloned plants gave a better yield in terms of fresh fruit bunch (FFB) and oil extract than seeded plants (Khoo *et al*., 1999). Somatic embryos induced from young leaf culture of oil palm ortet is an important material for oil palm propagation. Although they could be induced directly or indirectly by the use of dicamba (Te-chato *et al*., 2002), the conversion rate of somatic embryos to plantlets is quite low. Promoting germination of somatic embryo is of great importance in commercial plantation. Many authors have reported that various sources of carbon such as glucose, fructose, manitol and sorbitol play an important role in the germination of somatic embryos in asparagus (Mamiya and Sakamoto, 2000), cucumber (Lau and Sako,1995). Sako and Lou (1995) reported that a high concentration of sucrose (0.25 or 0.50 M) could enhance germination of somatic embryos in cucumber. The strength of MS medium is another factor that influence germination of somatic embryos. The study by Mamiya and Sakamoto (2000) showed that the highest germination of asparagus somatic embryos was obtained in double strength MS medium. In the case that the roots were not formed from shoots simultaneously they were transferred to half strength liquid MS medium supplemented with 0.03 M BA (6-Benzyladenine) and 0.06 M NAA (α-Napthaleneacetic acid) (Te-chato, 1998). To date, the effects of various carbon sources in inducing germination of somatic embryos or root induction in date palms (Tisserate and De-Mason, 1980) have been elucidated. Here, we report the effects of carbon sources and strength of MS-medium on germination of somatic embryo in oil palm.

Materials and Methods

Plant material

Somatic embryos of oil palm (at haustorium stage) induced from young leaves of elite clone on MS medium supplemented with 1-5 mg/l dicamba, 3% sucrose and 200 mg/l ascorbic acid were used. The pH of medium was adjusted to 5.7 and cultures maintained at 26±4ºC under 14 h photoperiod at 1,300 lux illumination and subcultured every 4 weeks.

Germination of somatic embryos

Haustorium-staged embryos were germinated on various strengths of MS medium (full, ½ and 1/5 strength). Each strength was supplemented with sucrose, glucose, fructose, mannitol or sorbitol at the concentrations of 0.1, 0.2 and

0.3 M. All cultures were grown under 14 h photoperiod, 1,300 lux illumination. After 3 months the germination percentage or shoot/root development was recorded.

Root induction

In cases where only shoots were produced from the somatic embryo and there was no development of roots, the shoots were transferred to full, ½ or 1/5 strength MS medium supplemented with 0.2 or 0.3 M sucrose, or sorbitol at a concentration of 0.3 M, 1/5 strength MS medium was used in combination with 0, 0.03, 0.05, 0.08, 0.16 M of sucrose. At 4 weeks of culture the percentage of root formation was examined.

Results

Germination of somatic embryos

Types and concentrations of sugar as carbon sources with reduced strength of MS medium resulted in development of a large number of secondary somatic embryos. The secondary embryos were initiated at basal part of primary embryo when cultured on germination medium for 2 weeks (Figure 1A) and could be multiplied in this medium (Figure 1B). Some embryos germinated only shoot after being cultured on this medium for 2-3 months (Figure 1C). MS medium supplemented with 0.2 M sorbitol gave the highest germination rate of embryos on germination medium (Table 1). The highest percentage and number of shoot germination was 40% and 2.75 shoots/explant (data not shown). Size of the shoots ranged from 0.5-0.8 cm in height, depending on strength of the medium. A 1/5 strength medium gave the smallest shoots. However, size of shoots increased when they were transferred to MS medium without plant growth regulator for 1-2 months. The highest shoots resulted from culturing on a shoot germination medium supplemented with sorbitol. Some somatic embryos from media supplemented with sorbitol produced roots after being transfered to hormone-free medium. These results indicated that high concentration of sucrose and lower strength media promoted root formation (Figure 1D).

Roots induction

Excised shoots produced the highest root at 31.25% when they were cultured on MS medium supplemented with 0.2 M sucrose for 1-2 months (Table 2, Figure 2). Reduction the strength of culture medium decreased percentage of root formation. Combination of sorbitol with sucrose gave the lower result than having 0.2 M sucrose alone.

Discussion

Sugar added in culture medium does not only act as a carbon source but also plays a role in osmotic regulation of water stress. Osmoticum increased the stress (water stress) of somatic embryo germination, inducing shoots and/or roots from those embryos of oil palm. Plant growth regulators were not used in the present study to avoid plantlets variation since a high concentration of plant growth regulator, especially 2,4-dichloropheneoxyacetic acid (2,4-D) and gibberellic acid (GA_3) can cause variation of *in vitro* plantlets. Nwanko and Krikorian (1983) reported that GA_3 at concentration only 1 mg/l could cause leaf curl and inhibited root induction of oil palm. In this study we found that sorbitol was a suitable osmoticum for shoot and root induction in oil palm. Not only shoots could be produced from somatic embryo, but also roots were formed simultaneously. Vespasiano and Wagner (2003) reported that osmoticum affected embryogenesis and plant regeneration, and sucrose promoted root formation in all concentrations. However, increasing the concentration of sucrose (0.2-0.3 M) and sorbitol (0.3 M) could enhanced phenolic compound formation. In addition, a high concentration of osmoticum promoted leaf blight similar to the effects of water stress. The plants died after being cultured on this medium for 2-3 months. So, completed plants should be transferred in hardening materials 2 weeks after induction of roots. Vespasiano and Wagne (2003) reported that concentrations of osmoticum alters osmotic potential

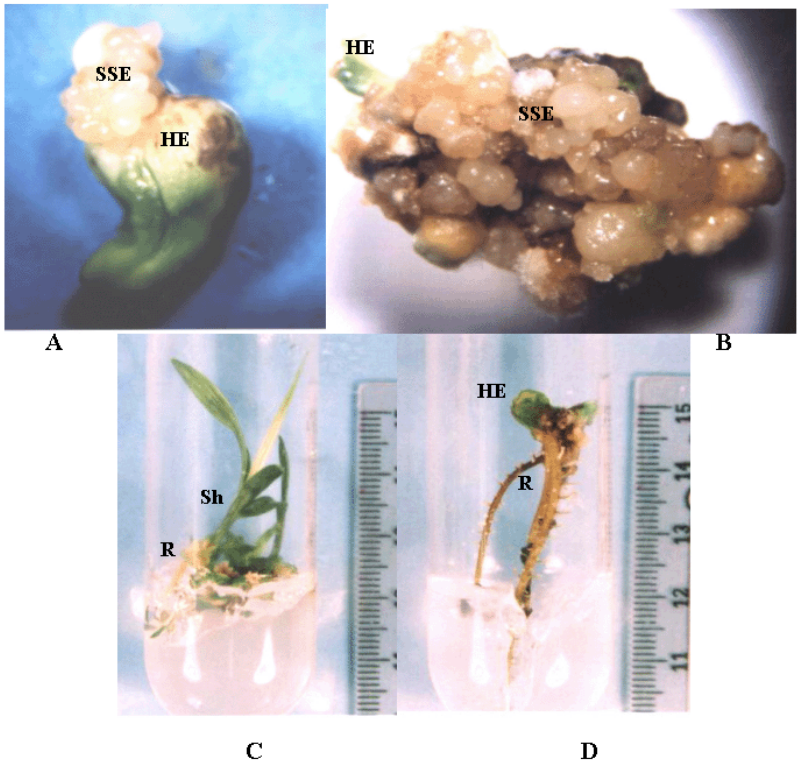

Figure 1. Germination of haustorium embryos (HE) of oil palm in germination medium. A, B: secondary somatic embryos (SSE) initiation. C: germination of SE (shoot, Sh with roots, R) in sorbitol-containing medium. D: Only root formation on a high concentration of sucrose.

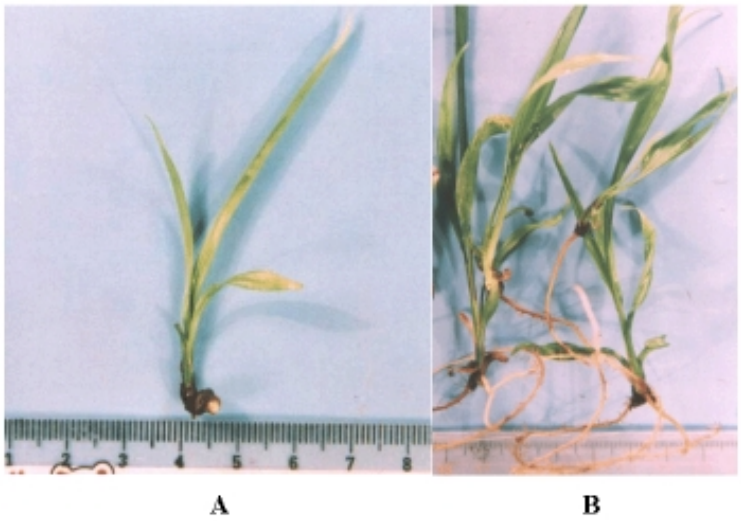

Figure 2. Shoot of oil palm was induced on half strength MS medium supplemented with 0.3 M sorbitol for 3 months (A). Completed plantlets of oil palm were induced on root induction medium for 4 weeks (B).

Table 1. Effect of kind and concentration of sugar and strengths of MS medium on germination and root or shoot formation of oil palm after culturing embryos for 3 months.

Carbon Source	Strength of MS Medium	Concentration (Molar)	No. secondary somatic embryo (embryo)		Shoots (%)	Roots (%)
			All	Haustorium (%)		
Sucrose	1	0.1	22.0	10.09	0	30
		0.2	15.40	10.38	20	40
		0.3	7.80	14.23	10	20
	½	0.1	10.60	24.52	30	20
		0.2	10.10	14.54	0	40
		0.3	11.44	17.48	0	50
	1/5	0.1	6.60	24.24	10	20
		0.2	4.00	25	0	50
		0.3	6.20	17.74	0	20
Fructose	1	0.1	6.67	44.97	0	0
		0.2	2.4	54.16	0	20
		0.3	3.8	44.73	0	0
	½	0.1	7.0	32.85	0	10
		0.2	5.2	36.53	0	0
		0.3	1.1	90.9	0	40
	1/5	0.1	3.2	62.5	0	40
		0.2	2.9	44.82	0	10
		0.3	1.0	100	0	30
Glucose	1	0.1	16.67	38.63	10	0
		0.2	17.0	11.05	10	0
		0.3	19.3	12.43	0	10
	½	0.1	14.6	21.9	0	0
		0.2	17.25	7.97	0	
		0.3	8.8	13.6	10	10
	1/5	0.1	6.22	23.15	0	10
		0.2	7.0	27.71	0	0
		0.3	15.2	7.23	0	0
Mannitol	1	0.1	0	0	0	0
		0.2	0	0	0	0
		0.3	0	0	0	0
	½	0.1	0	0	0	0
		0.2	0	0	0	0
		0.3	0	0	0	0
	1/5	0.1	0	0	0	0
		0.2	0	0	0	0
		0.3	0	0	0	0
Sorbitol	1	0.1	21.55	21.11	35	12.5
		0.2	11.55	27.87	40	11.11
		0.3	2.22	45.04	0	20
	½	0.1	11.16	41.75	22.22	22.22
		0.2	3.71	46.09	22.22	12.5
		0.3	5.12	58.59	22.22	14.28
	1/5	0.1	7.25	27.58	20	25
		0.2	3.71	46.09	0	22.22
		0.3	4.99	31.11	0	44.4

Table 2. Effect of concentration of sugar and strength of MS medium on root induction from excised shoots of oil palm.

Concentration (M)		Strength	Roots (%)
Sorbitol	Sucrose		
0.3	0	1/5	9.09
0.3	0.03	1/5	26.67
0.3	0.05	1/5	18.75
0.3	0.08	1/5	18.75
0.3	0.16	1/5	0
-	0.2	1	31.25
-	0.2	½	13.33
-	0.2	1/5	0
-	0.3	1	0
-	0.3	½	10
-	0.3	1/5	9.52

value of culture media which affects the somatic embryogenesis of plants. The addition of activated charcoal (AC) to the medium has proven useful for germination of somatic embryos in many cultures, for example *Phoenix dactylifera* (Tisserate and DeMason, 1980), *Averrhoa Carrambola* (Litz and Conover, 1980), and *Acacia nilotica* (Garg *et al*., 1996). Fridborg *et al*. (1978) showed that AC adsorbs a number of compounds, including auxins and culture metabolites which often inhibit specific developmental stages of somatic embryos. In the present study, it was observed that AC showed inhibitory effect and the embryos did not approach maturation, nor germination of SE/SSE (unpublished data).

Varying strength of nutrient components of the media have been shown to markedly influence the embryogenesis process in many plant species and suitable medium composition should be investigated for embryo induction, development, maturation and conversion. Park *et al*. (2005) reported that 1/3 strength MS hormone-free medium with 60 g/l sucrose was suitable for embryo induction from root segment. Mamiya and Sakamoto (2000) reported the highest germination of asparagus somatic embryos in double strength MS medium. In the present study, normal or basal MS with dicamba was preferred for somatic embryo induction from young leaves and root induction from excised shoots. In a previous study, Te-chato and Muangkaewngam (1992) found that MS medium modified by adding of vitamins improved root induction from single shoot. The most important finding of the current study was that there was higher developmental rate of roots in a high concentration of sucrose (0.2 M) containing medium or in stress conditions. To increase a yet higher root induction rate in further studies, we suggest using abscisic acid (ABA), or other osmotic chemical, such as polyethylene glycol (PEG) and high concentration of agar.

Conclusion

Sugar added in culture medium not only acts as carbon source but also plays a role in osmotic regulation of water stress, or osmoticum. Osmoticum enhanced germination of somatic embryos, and shoot and/or root development. The highest percentage of haustorium embryos and a large number of germinated embryos were obtained from the germination medium supplemented with sorbitol. However, the highest number of secondary somatic embryos was initiated on MS medium supplemented with 0.1 M sucrose. The percentage of root was highest (31.25%) when cultured on MS medium supplemented with 0.2 M sucrose.

Acknowledgement

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