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Effect of genotypes and auxins on callus formation from mature zygotic embryos of hybrid oil palms

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The effect of genotypes and auxins on percentage type of callus formation and type of callus from culturing mature zygotic embryos (MZEs) of six genotypes of *Elaeis quineensis* Jacq DxP; 366 (D) × 172 (P), 366 (D) × 72 (P), 366 (D) × 206 (P), 865 (D) × 206 (P), 110 (D) × 865 (P), 366 (D) × 777 (P) were studied. Callus formation was achieved on Murashige and Skoog (MS) medium supplemented with different concentrations of α-naphthaleneacetic acid (NAA) or 2,4-dichlorophenoxyacetic acid (2,4-D) or 3,6-dichloro-o-anisic acid (dicamba). The results revealed that all auxins were able to induce callus from culturing MZEs of some genotypes. The highest frequency of callus formation at 56.59% was obtained on the medium supplemented with 2.50 mg/l dicamba containing MS medium, significant difference to other kinds and concentrations of auxins. 366 (D) × 172 (P) gave the highest nodular callus formation and average number of nodular callus at 48.50% and 18.78±17.49 nodule/callus, respectively significant difference to others genotypes. Histological study revealed that origin of nodular callus obtained from 2,4-D containing medium arose from only epidermal cell layer while dicamba induced from both epidermal and vascular cells. NAA containing medium provided root like calluses which develop root primodia subsequent to root formation.

Key words: oil palm, genotype, auxin, mature zygotic embryo, callus

Introduction

Oil palm (*Elaeis quineensis* Jacq.) belongs to the family Arecaceae. It is a valuable economically important source of vegetable oil, the most traded vegetable oil in the international market, and is increasing used in the food industry (Corley and Tinker, 2003). In the world's supply, it takes second place after soybean. In 2007, the production of crude palm oil was 29.90 million tons (Palm oil, 2008). Based on data from the Food and Agricultural Organization (FAO), Thailand is respectively the world's fourth and third largest producer and exporter of palm oil. Consequently, palm oil production represents a significant and important part of the Thai economy. However, there is much

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room for improvement because the current average yield of palm oil in Thailand is only 2.8 tons per rai compared to 3.6 tons per rai in Malaysia (Basiron, 2002). Improvement in oil palm production, especially for oil yield, has generally been achieved via conventional program. As oil palm is normally propagated by seeds, this results in a high variation in the field and available sustainable genetic trait production in traditional breeding leading to the low palm oil yield. At present, micropropagation technique is applied for plant propagation in order to overcome some limitations (Aberlenc-Bertossi *et al.*, 1999; Rajesh, 2003). There have been previous reports of oil palm micropropagation through somatic embryogenesis (Te-chato, 1998a; Te-chato, 1998b; Te-chato *et al.*, 2003; Hilae and Te-chato, 2005). However, the most of callus cultures were successfull when cultured on high concentration of 2,4-D containing medium (Tisserat and Mason, 1980; Pannetier *et al.*, 1981; de Touchet, 1991). The use of high concentration of 2,4-D has been implicated as a cause of somaclonal variation in different species of plant including a vegetable oil (Eeuwens, 1978; Teixeira *et al.*, 1995; Chukwuemeka *et al.*, 2005). In order to reduce the variation from tissue culture-derived plant, protocols based on the use of lower levels of auxins should be considered. Hence, the objectives of present study are the effect of genotypes, various kinds and concentrations of auxins on callus formation from mature zygotic embryo culture of hybrid oil palms.

Materials and methods

Plant material

Mature fruits from six hybrids of oil palm; 366 (D) × 172 (P), 366 (D) × 72 (P), 366 (D) × 206 (P), 865 (D) × 206 (P), 865 (D) × 110 (P) and 366 (D) × 777 (P) were kindly provided by Assoc. Prof. Dr. Theera Eksomtramage (Agricultural Research Station, Khlong Hoi Khong, Hat Yai, Songkhla, Thailand). All seeds were extracted from the fruit, cracked by hammer and trimmed by pruning scissors to remove the excess kernel. Mature Zygotic embryos (MZEs) surrounded by kernel in cube of $3\times3\times3$ cm^3 were sterilized in 70% alcohol for two min followed by 20% (w/v) sodium hypochlorite together with two to three drops of Tween-20 for further 20 min. The cubes were then thoroughly washed in sterile water for three times. The embryos were excised from the cubes and cultured on media.

The effect of genotypes and auxins on percentage type of callus formation and type of callus

Sterilized MZEs were inoculated in culture tubes (25×150 mm) containing 10–15 ml of modified MS (Murashige and Skoog) medium supplemented with either 40 mg/l NAA or 2,4-D or dicamba at the concentration of 2.5 and 5.0 mg/l for type of callus formation. All media were adjusted pH to 5.7 with 0.1 N KOH before adding 0.7% agar, then autoclaved at 1.05 kg/cm^2, 121^{o}C for 15 min. The cultures were placed under light conditions of 3,000 lux illumination for 16 h photoperiod at 25±2^{o}C and subcultured every 4 weeks on the same medium component for 3 months.

Histological observation

For histological study, all types of callus formation were collected, fixed in FAA II solution (formalin: glacial acetic acid: 70% ethanol 5:5:90 v/v), dehydrated using an ethanol-tertiary butanol series for 24 h and embedded in Paraffin (Paraplast). Embedded tissues were sectioned at 6 μm and mounted on glass microscope slides. Paraffin was removed in a xylene-ethanol series; tissues were stained with saffranin and fast green. All sections were mounted with Permount and viewed under bright field illumination of compound microscope (Olympus.). Histological analysis was carried out on representative samples of the callus induced from different kinds and concentrations of auxins.

Data analyses

For experimental design and statistical analysis, completely randomized design (CRD) with 4 replicates (each replicate consist of 10 embryos) was performed. The percentage of cultures that produced callus, types of callus and number of the embryos per tube were recorded after 1 month for 3 months of culture by counting under a stereomicroscope (Nikon, SMZU). Data were analyzed using analysis of variance (ANOVA).

Results and discussion

The effect of genotypes and auxins on percentage type of callus formation and type of callus

MZEs of all genotypes swell at 10-14 days of culture and started to from callus at 4-5 weeks of culture from MZEs. After 6 weeks of subculture onto

various kinds and concentrations of auxins, almost auxins promoted callus formation form MZEs of all crosses. Four types of calluses could be distinguished; friable, compact, nodular and root-like calluses. The friable calluses were yellow, translucent and succulent. The compact calluses were muddy white and compact. The nodular calluses were yellow or pale yellow and compact. The root-like calluses were elongate in shape, white color and soft texture. Kinds and concentrations of auxins used in media had a significant effect on type of calluses. Characteristics of the callus obtained in NAA, 2,4-D and dicamba containing the medium were quite different. Dicamba provided a yellow compact callus (so called nodular callus) whereas 2,4-D gave both a white friable callus and a white elongative soft callus. For NAA, it could be not induced callus formation (Fig. 1). The highest frequency of callus formation was obtained on the medium supplemented with 2.50 mg/l dicamba containing MS medium, significant difference to other kinds and concentrations of auxins (Table 1). Maria and Heidi (2002) also reported that dicamba was effective for callus induction from culturing of wheat (*Triticum aestivum* L.). Similar result was also found in immature embryo culture of winter wheat (Carman *et al.*, 1988) and spring wheat cultivars (Hunsinguer and Schauz, 1987). Dicamba is promising auxins which has been reported to be an effective on promoting direct and indirect embryogenic callus induction from cultured mature zygotic embryo and young of leaf oil palm (Te-chato, 1998a). Time consumed for callus induction in culture medium supplemented with dicamba was shorter earlier than 2,4-D and NAA. Similar result was obtained from culturing young leaf of the same plant (Te-chato, 1998b; Te-chato *et al.*, 2003). In addition, 2,4-D containing medium was reported to induce nodular structure from epidermal cells of mature zygotic embryo while dicamba induced from both the epidermis and vascular tissues (Thawaro and Te-chato, 2007). In the present study three different synthetic auxins (NAA, 2, 4-D and dicamba) were compared with genotypes. The result of our experiments show that among genotypes tested, cross #58 obtained from combination of 366 (D) × 172 (P) gave the highest percentage of nodular callus formation at 48.50% and average number of nodular callus formation at 18.78±17.49 nodule/callus, significant difference to others genotypes when cultured on 2.50 mg/l dicamba (Table 2, Fig. 2.).

Histological observation

Histological study revealed that 2,4-D containing medium induced nodular structure from epidermal cells of mature zygotic embryo while dicamba induced from both the epidermis and vascular tissue after 4 weeks of culture. These cells were small, cytoplasm dense and compose of well stained

nucleus. They proliferated intensely which caused a rupture of the epidermis and hence emergence of the nodular structure at the surface of zygotic embryo (Fig. 3.). In case of NAA containing medium, nodular and elongative root-like structures (Fig. 4. A) arose from the epidermal layer of mature zygotic embryo (Figure 4B) and those structures developed to root primordial (Fig. 4. C). This type of development was also evident in nodular structure obtained from 2.5 mg/l 2,4-D containing medium. By the results in this investigation, it suggested that dicamba was the most potent plant growth regulator for nodular callus induction both qualitatively and quantitatively like those reported by Promchan and Te-chato (2007) and Te-chato and Hilae (2007). So, mass propagation of oil palm though tissue culture technique could be commercialized by this phytohormone.

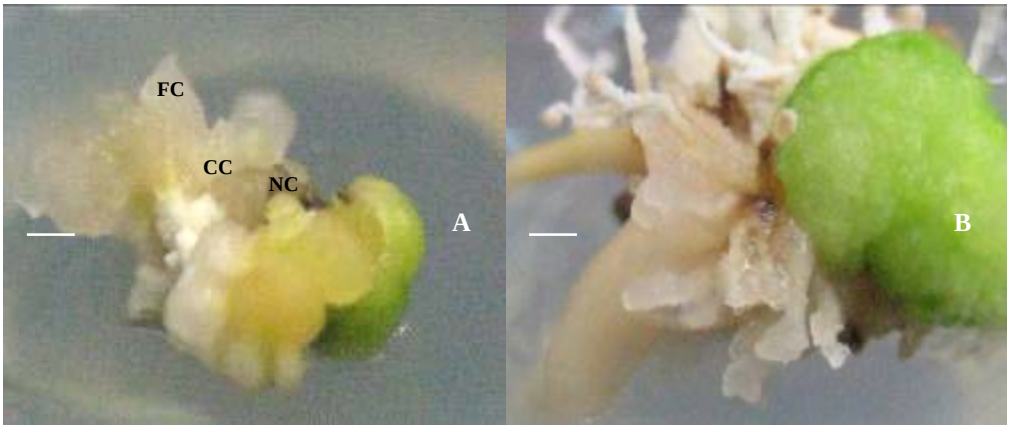

Fig. 1. Cluster of friable callus (FC), compact callus (CC), nodular calluses (NC) (A) and root-like structure (B) from MZEs of hybrid oil palms cultured on solid MS supplemented with various auxin for 3 months (bar = 2.50 mm).

Table 1. Effect of various kinds concentrations of auxin on percentage of callus formation from various hybrid oil palms after culture for 3 months.

Hybrid	Auxin	Concentration (mg/l)	Type of callus formation (%)			
			Friable callus	Compact callus	Nodular callus	Root like callus
366(D)×72(P)	NAA	40	15a	0b	2.50c	27.50bc
	2,4-D	2.50	2.50b	2.50b	37.50b	12.50c
		5	20a	12.50a	70ab	52.50ab
	Dicamba	2.50	17.50a	15a	87.50a	70a
		5	22.50a	12.50a	45b	22.50bc
Mean			15.50	8.50	48.50	37
366(D)×172(P)	NAA	40	15b	5b	2.50d	25a
	2,4-D	2.50	22.50b	7.50b	27.50b	10ab
		5	20b	7.50b	35b	0b
	Dicamba	2.50	50a	12.50a	65a	0b
		5	10b	12.50a	10c	0b
Mean			23.50	9	28	7
366(D)×206(P)	NAA	40	0c	0c	0c	0c
	2,4-D	2.50	23.57b	9.80b	39.22a	7.84b
		5	15ab	5c	20b	7.50b
	Dicamba	2.50	33.33a	17.65a	41.82a	15.69a
		5	28.33a	16.67a	41.18a	20a
Mean			20.05	9.82	28.44	10.21
865(D)×206(P)	NAA	40	0c	0c	0c	0b
	2,4-D	2.50	29.09b	8.18b	46.60a	11.82a
		5	35.29a	11.76a	39.22b	13.73a
	Dicamba	2.50	34.65a	11.65a	50.19a	15.84a
		5	29.13b	6.93b	42.57a	12.62a
Mean			25.63	7.70	35.72	10.80
865(D)×110(P)	NAA	40	0b	0b	0d	0b
	2,4-D	2.50	12.50a	7.50a	7.50c	10a
		5	12.50a	10a	40b	10a
	Dicamba	2.50	15a	7.50a	42.50a	15a
		5	12.50a	10a	12.50c	4.5ab
Mean			10.50	7	20.50	7.90
366(D)×777(P)	NAA	40	0c	0c	0d	0b
	2,4-D	2.50	17.50b	12.50b	37.50c	5a
		5	17.50b	12.50b	47.50ab	7.50a
	Dicamba	2.50	37.50a	20a	52.50a	0b
		5	25ab	10b	45b	0b
Mean			19.50	11	36.50	2.50
F-test			*	*	*	*
C. V. (%)			25.15	35.17	31.16	30.55

*Means followed by the same letter do not differ significantly (p<0.05).

Table 2. Effect of various kinds concentrations of auxin on average number of callus formation from various hybrid oil palms after culture for 3 months.

Cross	Auxin	Concentrations (mg/l)	Average number of callus / embryo	
			Nodular callus ± SD	Root like callus ± SD
366(D)×72(P)	NAA	40	3.82 ± 1.72c	2.36 ± 1.50c
	2,4-D	2.50	8.22 ± 4.15bc	1.60 ± 0.55bc
		5	7.93 ± 4.23bc	2.90 ± 1.54bc
	Dicamba	2.50	18.78 ± 17.49a	5.22 ± 4.60a
		5	11.75 ± 5.06ab	4.03 ± 2.38ab
Mean			10.10 ± 4.64	3.22 ± 2.11
366(D)×172(P)	NAA	40	3.54 ± 1.90b	1.55 ± 0.82a
	2,4-D	2.50	4.27 ± 1.09b	0.44 ± 0.33b
		5	6.46 ± 6.19ab	0.00 ± 0.00c
	Dicamba	2.50	17.31 ± 8.87a	0.00 ± 0.00c
		5	11.57 ± 9.47ab	0.00 ± 0.00c
Mean			8.63 ± 3.78	0.40 ± 0.23
366(D)×206(P)	NAA	40	0.00 ± 0.00b	0.00 ± 0.00b
	2,4-D	2.50	15.95 ± 11.35a	1.75 ± 0.54a
		5	10.63 ± 8.19a	3.33 ± 1.39a
	Dicamba	2.50	17.67 ± 14.33a	2.75 ± 0.77a
		5	16.17 ± 10.88a	3.76 ± 1.02a
Mean			12.08 ± 8.95	2.32 ± 0.74
865(D)×206(P)	NAA	40	0.00 ± 0.00c	0.00 ± 0.00b
	2,4-D	2.50	14.25 ± 6.78a	1.00 ± 0.02a
		5	12.60 ± 5.11b	1.57 ± 0.79a
	Dicamba	2.50	15.09 ± 7.02a	2.13 ± 1.03a
		5	14.15 ± 6.55a	1.77 ± 0.78a
Mean			11.29 ± 5.09	1.29 ± 0.52
865(D)×110(P)	NAA	40	0.00 ± 0.00c	0.00 ± 0.00b
	2,4-D	2.50	9.06 ± 7.15b	1.50 ± 0.17a
		5	6.88 ± 3.14b	1.25 ± 1.02a
	Dicamba	2.50	12.68 ± 6.31a	1.13 ± 1.06a
		5	7.22 ± 2.69b	1.00 ± 0.17a
Mean			7.17 ± 3.86	0.98 ± 0.48
366(D)×777(P)	NAA	40	0.00 ± 0.00c	0.00 ± 0.00b
	2,4-D	2.50	5.48 ± 2.34b	1.50 ± 1.22a
		5	5.71 ± 2.39b	1.33 ± 1.15a
	Dicamba	2.50	11.43 ± 3.39a	0.00 ± 0.00b
		5	9.00 ± 2.84b	0.00 ± 0.00b
Mean			6.32 ± 2.19	0.57 ± 0.47
F-test			*	*
C. V. (%)			22.06	20.09

*Means followed by the same letter do not differ significantly (p<0.05).

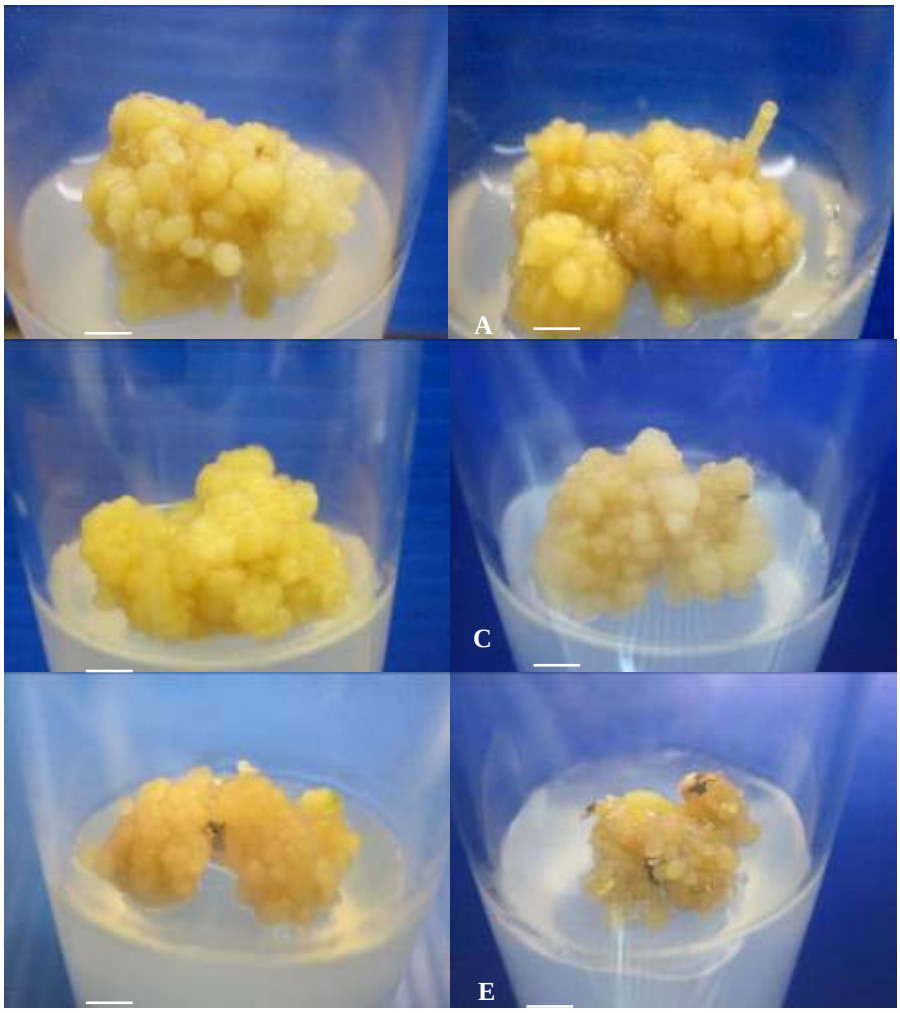

Fig. 2. Nodular callus induction from MZEs of cross #77 (A), cross #58 (B), cross #118 (C), cross #119 (D), cross #130 (E) and cross #137 (F) cultured on solid MS supplemented with 2.5 mg/l dicamba (bar = 2.50 mm).

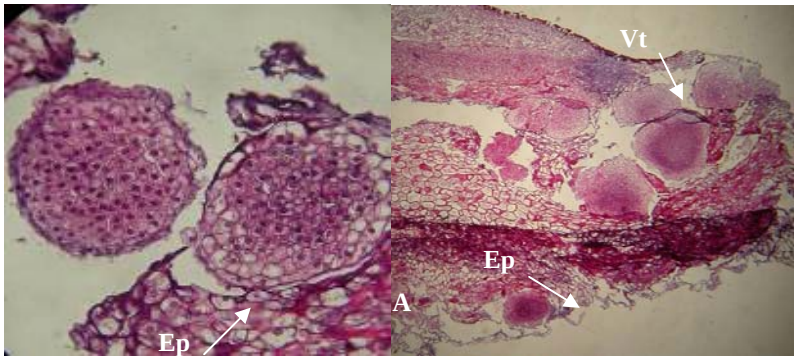

Fig. 3. Histologicai study of callus in 2, 4-D (A) or dicamba (B) containing medium. A: showed development of nodular structure from epidermis (Ep) only. B: showed development of nodular structure from both epidermis (Ep) and vascular tissue (Vt) (bar = 50 µm).

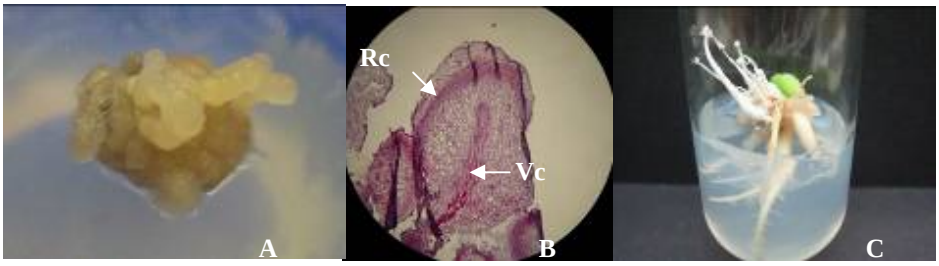

Fig. 4. Morphological (A) and histological study (B) of callus in NAA or 2,4-D containing medium and morphological structure of not on the callus (C). Rc: root cap, Vc: vascular cambium. (bar = 50 µm).

Conclusion

The results of our experiment indicated that 2.50 mg/l dicamba gave the highest frequency of callus formation, significant difference to other kinds and concentrations of auxins. Cross #58 obtained from combination of 366 (D) × 172 (P) gave the highest nodular callus formation (48.50%) and average number of nodular callus formation (18.78±17.49 nodule/callus), significant difference to others genotypes. Histological study revealed that origin of nodular callus obtained form 2,4-D containing medium was from only epidermis while dicamba induced both epidermis and vascular tissues. NAA containing medium provided root like calluses which develop root primodia subsequent to root formation.

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