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Effect of culture medium and genotype on germination of hybrid oil palm zygotic embryos

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ABSTRACT: Nutritional requirements for germination of mature zygotic embryos of four genotypes of *Elaeis guineensis* Jacq. Dura × Pisifera were assessed. A factorial experiment was conducted with four genotypes from separate crossing events and three culture media to show Interactions between genotypes and culture media on seedling germination ($p < 0.05$). The highest frequency of normal seedling germination was facilitated by 1/2 Murashige and Skoog (MS) medium (29.2%), followed by MS (15.0%) and Blaydes (12.9%). Speed of shoot formation followed a similar trend. Roots were induced most rapidly in 1/2 MS medium, followed by Blaydes and then MS. Root induction was similar in 1/2 MS medium (2.29%) and Blaydes medium (1.25%) but only significantly higher than in MS (0.00%).

KEYWORDS: oil palm, genotype, culture medium, zygotic embryo, germination

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is the source of the most sought-after edible oil in the world market[1]. In Thailand, recently a growing amount of palm oil has been used for the production of biodiesel. For biodiesel production, a high yield crop is needed, and the large amount of oil produced by the oil palm fruit makes this species highly suitable. Currently, all major commercial oil palms are F1 hybrids between selections with small or no kernels (pisifera) and large thick kernels (dura). The hybrids (tenera) show high variations in oil yield with the best plants yielding 40% more than average. As a monocotyledonous species with a single growing apex, the plant cannot be multiplied vegetatively[3]. Commercial propagation of oil palm through tissue culture is widely used[4]. Plant regeneration of oil palm through culture in vitro has been reported by several researchers[5–7]. A reliable and efficient procedure for propagation of elite in vitro clones has the potential to increase yields significantly.

Earlier reports from our laboratory were based on regeneration from leaf explants using dicamba in oil palm tissue culture[8–10]. The establishment of plant regeneration by embryogenesis is satisfactory in oil palm. To date, the only explant allowing reproducible results with a wide range of genotypes is the zygotic embryo, although the technique requires considerable nutritional requirements and genotypes for seedling induction. Hence, the objective of the present study is to examine the effect of three kinds of culture medium and genotypes on seedling induction from zygotic embryo culture of hybrid oil palms.

MATERIALS AND METHODS

Plant material

Mature oil palm fruits of four hybrid oil palms were kindly provided by Dr Theera Eksomtramage (Agricultural Research Station, Hat Yai). These intraspecific crosses were conducted between dura (D) and pisifera (P) types and were collected at 180 days after pollination. The hybrid fruit used in this experiment included: genotype 77 (cross 366 (D) × 172 (P)), genotype 58 (cross 366 (D) × 72 (P)), genotype 118 (cross 366 (D) × 206 (P)) and genotype 119 (cross 865 (D) × 206 (P)). The fruit was cracked with a hammer and trimmed with pruning scissors to remove excess kernel. Zygotic embryos were sterilized in 70% alcohol for 2 min, followed by 20% (w/v) sodium hypochlorite together with 2–3 drops of Tween-20 for 20 min. The cubes were then thoroughly washed in sterile water 3 times. The embryos were excised from the cubes and cultured on culture medium.

Effect of genotype and culture media on germination of zygotic embryos of oil palms

Sterilized mature zygotic embryos of the four genotypes were grown in culture tubes containing 10–15 ml of one of the three culture media: Murashige

and Skoog (MS), 1/2 MS, and Blaydes (BL). The pH of all the culture media was adjusted to 5.7 with 0.1 N NaOH or HCl before adding (0.75%) agar and autoclaving at 10^5 Pa, 121 °C for 15 min. The cultures were placed under light conditions (3000 lux illumination for 16 h photoperiod) at 25 ± 2 °C and subcultured every 4 weeks for 3 months.

Growth measurements and statistical analysis

The experimental design for germination involved two factorial completely randomized designs with 4 replications (each replication consisted of 10 embryos). There were 4 levels of the first factor (genotypes) and 3 levels of the second factor (culture media). The percentage of cultures that produced shoots, roots, and both shoots and roots formations (i.e., normal seedling growth) were recorded after 1 month of culture. The numbers of leaves and roots, leaf and root length, and height of seedling were noted and compared every month for 3 months. The speed of germination index was calculated and modified according to Santipracha[11]. The average number of leaves and roots were assessed by counting well developed plantlets. Data were analysed using analysis of variance (ANOVA version 14.0). Means were separated by Duncan's multiple range tests at 5% significance level. Speed of shoot formation index (SSI), speed of root formation index (SRI), and speed of shoot and root formation index (SSRI) were obtained from

$$\mathrm{SSI} = \sum \frac{S_t}{t}, \quad \mathrm{SRI} = \sum \frac{R_t}{t}, \quad \mathrm{SRRI} = \sum \frac{U_t}{t},$$

where S_t, R_t, and U_t are the number of shoots, roots, and shoots with roots formed on day t.

RESULTS

Swelling of the zygotic embryo of all genotypes was observed at 7–10 days of culture and the shoots initiated at 14 days of culture. Each medium promoted seedling germination from zygotic embryos of all genotypes after 1 month of culture. Three main types of growth responses were observed: induction of shoot only, root only, and both shoot and root. The distinctive effects on growth were more apparent after the second transfer to fresh medium. After 3 months of subculture on various kinds of medium, there were healthy and normal seedlings of all genotypes.

The highest shoot induction (29.7%) and shoot induction index (6.89) was found for genotype 58 on 1/2 MS medium (Table 1). Genotype 58 gave the highest mean shoot induction (21.1%), which was significantly higher than the other genotypes. A similar trend in genotype response was found for shoot induction index. Embryos grown in 1/2 MS medium had similar shoot induction (19.0%) to MS medium (16.9%) but was significantly higher than for embryos grown in BL medium (11.0%). A similar trend in medium response was found for shoot induction index. There was an interaction between genotypes and culture media on

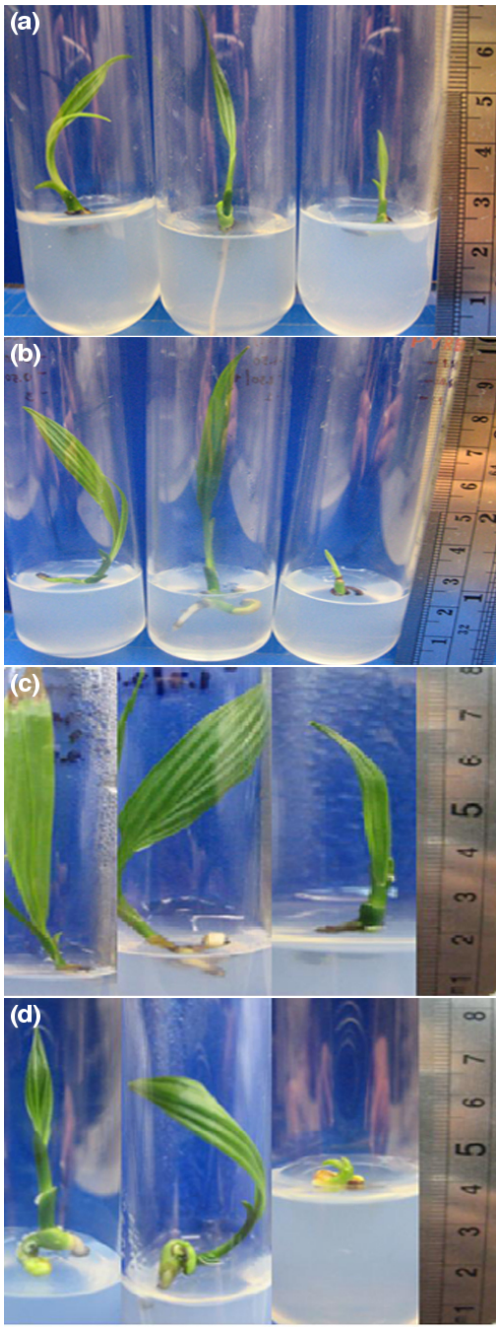

Fig. 1 Germination of mature zygotic embryos on various culture media for 3 months. Genotypes: (a) 77, (b) 58, (c) 118, (d) 119.

Table 1 Effect of genotypes and culture media on the percentage of shoot induction and SSI in mature zygotic embryo of oil palm after 3 months culture in 10–15 ml MS, 1/2 MS, or Blaydes medium and subcultured to fresh medium after 4 weeks.

Genotypes	Shoot induction (%)				SSI			
medium:	MS	1/2 MS	BL	[Mean]	MS	1/2 MS	BL	[Mean]
77	15.00^{bcde}	11.67^{bcde}	10.83^{cde}	12.50^{BC}	3.67^{bc}	2.39^{bc}	2.77^{bc}	2.94^{B}
58	20.83^{abc}	29.17^{a}	13.33^{bcde}	21.11^{A}	3.06^{bc}	6.89^{a}	5.06^{ab}	5.00^{A}
118	9.17^{de}	15.83^{bcde}	7.50^{e}	10.83^{C}	1.83^{c}	3.67^{bc}	1.61^{c}	2.37^{B}
119	22.50^{ab}	19.17^{abcd}	12.50^{bcde}	18.06^{AB}	4.50^{abc}	4.11^{abc}	2.61^{bc}	3.74^{AB}
Mean	16.88^{AB}	18.96^{A}	11.04^{B}		3.27^{B}	4.27^{A}	3.01^{B}	
F-test / C.V. (%)	* / 22.11				* / 38.72			

C.V. (%) = coefficient of variation

For Tables 1–3, different lower case letters indicate significant differences between genotypes between the four media at $p < 0.05$. Different uppercase letters include significant differences between medium (row-wise comparison) or genotypes (column-wise comparison) at $p < 0.05$.

both shoot induction and shoot induction index ($p \leqslant 0.05$). The highest shoot induction values for embryos grown on 1/2 MS and BL medium were produced by genotype 58. However, the highest shoot induction on MS medium was produced by genotype 119. A similar trend in genotype response was found for shoot induction index.

The highest root induction (5.00%) and root induction index (0.83) was found for genotype 77 on 1/2 MS medium (Table 2). Genotype 118 gave the highest mean root induction (2.22%), which was significantly higher than the others. A similar trend in genotype response was found for root induction index. The highest root induction for embryos grown on 1/2 MS were produced by genotype 77, however, the highest shoot induction on BL medium was produced by genotype 118. No roots were produced by embryos grown on MS medium.

The highest shoot+root induction (7.50%) and shoot+root induction index (1.00) was found for genotype 58 on 1/2 MS medium (Table 3). Genotype 58 gave the highest mean shoot+root induction (4.71%). There were no significant differences between genotypes for shoot+root induction index. Embryos grown in 1/2 MS medium had significantly higher shoot+root induction (2.71%) than in other media. There were no significant differences between media for shoot+root induction index. There was no interaction between genotype and culture media for shoot+root induction and no significant differences in the index ($p \leqslant 0.05$).

The average height (5.38 cm), number of leaves (3.96) and leaf length (3.60 mm) was highest for genotype 58 (Table 4). Genotype 58 also produced the most roots (0.39). Roots were longest in genotype 118. There was an interaction between genotype and culture medium for seedling induction measurements. Embryos of genotype 77 were the tallest with the highest number and longest leaves when grown on MS medium. However, these embryos did not develop roots. BL medium produced embryos in genotype

Table 2 Effect of genotypes and culture media on the percentage of root induction and speed of root induction index (SRI) in mature zygotic embryo of oil palm after 3 months culture.

Genotypes	Root induction (%)				SRI			
	MS medium	1/2 MS medium	BL medium	Mean	MS medium	1/2 MS medium	BL medium	Mean
77	0.00^{c}	5.00^{a}	0.00^{c}	1.67^{AB}	0.00^{c}	0.83^{a}	0.00^{c}	0.28^{ns}
58	0.00^{c}	0.83^{bc}	0.83^{bc}	1.11^{AB}	0.00^{c}	0.17^{bc}	0.17^{bc}	0.11
118	0.00^{c}	4.17^{ab}	2.50^{abc}	2.22^{A}	0.11^{bc}	0.67^{ab}	0.28^{abc}	0.35
119	0.00^{c}	0.83^{bc}	0.00^{c}	0.28^{B}	0.00^{c}	0.00^{c}	0.11^{bc}	0.04
Mean	0.00^{B}	2.71^{A}	1.11^{AB}		0.03^{B}	0.42^{A}	0.14^{B}	
F-test / C.V. (%)	* / 64.62				* / 44.38			

ns is not significantly different at $p < 0.05$.

Table 3 Effect of genotypes and culture media on the percentage of shoot and root induction and speed of shoot and root induction index (SSRI) in mature zygotic embryo of oil palm after 3 months culture.

Genotypes	Shoot + root induction (%)				SSRI			
	MS medium	1/2 MS medium	BL medium	Mean	MS medium	1/2 MS medium	BL medium	Mean
77	0.00^{c}	0.83^{c}	0.00^{b}	0.28^{B}	0.00	0.11	0.00	0.04^{ns}
58	2.50^{a}	7.50^{a}	2.50^{a}	4.17^{A}	0.33	1.00	0.33	0.55
118	0.00^{c}	2.50^{b}	0.00^{b}	0.83^{B}	0.00	0.33	0.00	0.11
119	0.83^{b}	0.00^{c}	0.00^{b}	0.28^{B}	0.67	0.00	0.00	0.22
Mean	0.83^{B}	2.71^{A}	0.63^{B}		0.25	0.36	0.08	
F-test / C.V. (%)		* / 21.79				ns / 63.82		

Table 4 Effect of genotypes and culture media on height, number of leaves, length of leaves, number of roots and length of roots (seedling induction measurements) in mature zygotic embryo of hybrid oil palm after 3 months culture.

Genotypes	Medium	Seedling induction				
		Mean of height (cm)	Mean no. of leaves	Mean leaf length (mm)	Mean no. of root	Mean root length
77	MS	3.78^{bc}	2.75^{bc}	2.23^{abcd}	0.00^{b}	0.00
	1/2 MS	2.84^{bc}	2.05^{ab}	0.75^{cd}	1.15^{a}	0.58
	BL	1.79^{c}	1.04^{b}	0.29^{d}	0.00^{b}	0.00
	Mean	2.80^{C}	1.95^{B}	1.09^{C}	0.38^{ns}	0.19^{ns}
58	MS	5.80^{ab}	3.42^{ab}	4.16^{ab}	0.08^{b}	0.02
	1/2 MS	7.11^{a}	4.88^{a}	4.64^{a}	1.08^{a}	0.60
	BL	3.21^{bc}	3.58^{ab}	1.99^{abcd}	0.00^{b}	0.00
	Mean	5.38^{A}	3.96^{A}	3.60^{A}	0.39	0.20
118	MS	4.67^{abc}	2.81^{abc}	2.23^{abcd}	0.08^{b}	0.26
	1/2 MS	4.42^{abc}	3.17^{ab}	2.02^{abcd}	0.58^{ab}	0.63
	BL	2.95^{bc}	1.00^{b}	0.83^{cd}	0.17^{b}	0.08
	Mean	4.01^{B}	2.32^{AB}	1.73^{BC}	0.28	0.32
119	MS	7.25^{a}	4.42^{a}	2.89^{abcd}	0.25^{b}	0.08
	1/2 MS	5.21^{ab}	4.45^{a}	3.47^{abc}	0.33^{b}	0.21
	BL	3.51^{bc}	2.63^{ab}	1.46^{bcd}	0.00^{b}	0.00
	Mean	5.32^{A}	3.83^{A}	2.61^{AB}	0.19	0.10
F-test / C.V. (%)		* / 35.44	* / 55.56	* / 65.35	ns / 120.27	ns / 175.21

77 that were the shortest with the least number and shortest leaves. Root production in genotype 77 was only observed when grown on 1/2 MS medium. A similar trend was observed for genotype 118. However, most of the leaves developed when embryos were grown on 1/2 MS medium. Embryos of genotype 58 produced the tallest plantlets with the highest number and longest leaves and highest number and longest roots on 1/2 MS medium. A small number of roots were observed on embryos grown MS medium. However, no roots developed when grown in BL medium. A similar trend was observed for genotype 119. However, the tallest embryos were produced when grown on MS medium.

DISCUSSION

On the basis of culture, MS is widely used as a basal medium for proliferation in oil palm tissue culture[12–14] and germination tests[15]. Culture media play a significant role in germination. Generally, germination of seedling requires only the basal medium without plant growth regulators as the embryo is capable of synthesizing plant growth regulator itself. In the case of culturing zygotic embryos, only basic nutrients for germination are necessary due to removal of food storage tissue.

The results from this study showed that 1/2 MS medium was the most suitable among the culture media tested and was sufficient for the germination of

seedling. MS medium has a higher concentration of nutritional constituents and produced similar effects on leaf development but inhibited root growth. Similar results were obtained in the seagrasses *Halophila ovalis* and *Posidonia coriacea*[16]. Many authors have reported the use of basal MS for culturing of mature zygotic embryo (MZEs) of various horticultural crops[17–20]. Decreasing the concentration of MS to half strength (1/2 MS) was also reported to be suitable for promoting germination of MZEs in oil palm[21].

In addition, Y3 medium has been reported to germinate oil palm MZEs[22]. However, supplementing the culture medium with 0.05% activated charcoal gave the best seedling formation. By comparison, Y3 lacks NH_4NO_3, KH_2PO_4, and glycine, which are present in MS medium. The concentration of the remaining components ($CaCl_2$, $MgSO_4$, $MnSO_4$, $ZnSO_4$, H_3BO_4, KI, Na_2MoO_4, $CuSO_4$, $CoCl_2$, Na_2-EDTA, $FeSO_4$, nicotinic acid, and pyridoxine-HCl) are lower than in MS medium[23,24]. Those components and their concentration have been reported to be important for the germination of barley (*Hordeum vulgare* L. var. BL-2)[25].

The concentration of KNO_3 is higher in Y3 than in MS. KNO_3 is used primarily on high value crops. It has a neutral effect on pH and is a source of soluble nitrogen and potassium. It has an important function in the osmotic regulation of the cell, controlling the influx of other compounds[26]. As a consequence of potassium uptake, nitrate use increases, promoting growth. Potassium nitrate was found to be important in the germination of plum (*Prunus domestica* L.)[27] while some banana has a preference for NH_4NO_3[28].

MS medium has a high concentration of NH_4NO_3, which is a source of NH_4^+ and NO_3^{2+}. Nitrate-N induces the activity of nitrate reductase enzyme that reduces nitrate to ammonium, which is then incorporated into proteins[29,30]. Additionally, the form of inorganic nitrogen and ammonium to nitrate ratio has been reported to greatly affect the growth and differentiation of cultured tissues[31,32]. Nevertheless, with a high NH_4^+, the uptake of cations is reduced and replaced instead by the uptake of an anion such as phosphorus with an accompanying release of an H^+ equivalent, which could lead to a more acidic pH and could be toxic to cells[27]. Glycine, present in MS medium, is also a source of organic nitrogen which has been reported to enhance plant regeneration in maize[33].

MS medium is the widely used standard medium for cultured tissues and has the sufficient nutrient requirements of oil palm germination. MZEs of oil palm cultured on full strength MS hormone-free medium gives a higher percentage of germination than Y3 hormone-free medium[34]. In addition, MZEs crosses cultured on full strength MS medium give the highest percentage of germination[35]. Therefore Y3 was considered to be unsuitable for the present study.

The use of a solid rather than liquid was decided as the physical state of the culture media affected plant development. The liquid medium induced the regeneration of adventitious plantlets whereas the solid medium induced the growth of plants[36]. Similarly, it has been reported that solid medium induces callus formation, whereas liquid medium induces the development of the differentiated callus in four species of *Porphyra*, *Laminaria angustata*, and *Dictyosiphon foeniculaceus*[37].

In this study, embryos germinated on Blaydes medium had the lowest shoot growth and moderate root growth compared with 1/2 MS and MS medium. Singlaw[38] also found that MZEs in Blaydes medium had a lower percentage of germination than in MS medium. The concentration of micronutrients is lower in Blaydes medium than in MS medium[39,40]. Macronutrient components and concentrations have been reported to be important for the germination of quince[41].

Genotypes also played an important role in germination of embryos. In this study, genotypes 58 produced the highest shoot induction and, shoot+root induction whereas genotypes 118 produced the highest root induction when cultured on 1/2 MS medium without plant growth regulator. Germination of indica rice (*Oryza sativa*) is reported to depend upon genotypes[42]. It was shown that the karnal local cultivar cultured on MS medium gave the best frequency germination at 88%, which is significantly higher than for IR-72 (47.5%) and IR 54 (25%) breeding lines. Genotype affects the germination of oil palm as well. The highest percentage of germination has been obtained in 366 (D) $\times$ 110 (P) cross combination after culturing on growth regulator-free MS medium when compared with 865 (D) $\times$ 110 (P) cross combination[43]. Considerably different genotype capacity has been observed to produce different response between individuals of a cultivar or species.

Some genotypes exhibit high germination capacity, while others are either recalcitrant or irresponsible[44,45]. Specific genes are probably involved in each stage of plant development or regeneration[46] (dedifferentiation, acquisition of competence, and induction). Furthermore, the size and vigour of seed or zygotic embryo is of great importance in the germination of oil palm. As larger seeds contain larger zygotic embryos of all crosses, healthy ger-

mination of seedlings is obtained[47]. Generally, the larger seeds have larger food storage and are more capable of synthesizing plant growth regulator than the small seeds. Vigorous seeds, which are generally assessed by germination test and demonstrated by germination speed index also have a silencing power for germination. Sanputavong and Te-chato[48] found that cross number 16 had more hybrid vigour than another crosses. The different responses might be due to different genotype capacity, and the size and vigour seed of oil palm. In conclusion, the key factors affecting the germination of oil palm were different genotypes or cross combinations and culture media which caused different responses on germination.

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