Journal of Agricultural Technology 2011 Vol. 7(2): 531-545
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Analysis of somaclonal variation of callus, somatic embryo and plant regeneration of *in vitro* oil palm (*Elaeis guineensis* Jacq.)

Sanputawong, S. and Te-chato, S.*

Department of Plant Science, Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Songkhla, 90112, Thailand.

Sanputawong, S. and Te-chato, S. (2011). Analysis of somaclonal variation of callus, somatic embryo and plant regeneration of *in vitro* oil palm (*Elaeis guineensis* Jacq.) Journal of Agricultural Technology 7(2): 531-545.

This study describes the plant regeneration from immature zygotic embryo (IZE) and verification of hybrid and somaclonal variation from oil palm genomic sequences. Simple sequence repeat (SSR) was used to assess the hybrid verification and somaclonal variation of the cross number 7 and 16 of hybrids oil palm. DNA from IZE was isolated and detected by eight primers of SSR. Variation of somaclone obtained through somatic embryogenesis was detected at the callus, somatic embryo and leave of plantlet of the culture period. The result revealed that all SSR primers tested could amplify parental DNA. Primer EgCIR008 of SSR provided clear DNA patterns and could be used for verification the hybridity and somaclonal variation of both cross. The SSR markers can be used for clonal identification, monitoring line uniformity within clones. After transferring the calli to culture on MS medium supplemented with 1 mg/l dicamba for 2 months the embryogenic callus was formed from cross number 7. The highest percentage of embryogenic callus (2.94) and number of somatic embryo/callus formation (33.33) were obtained from cross number 7. Somatic embryos (SEs) at the final stages, called, haustorium embryos (HEs) was obtained from both crosses. Secondary somatic embryos (SSEs) were achieved on MS medium supplemented with 0.2 M of sorbitol and 200 mg/l ascorbic acid (As). Plantlet conversion rate at the highest average number of 0.8 was obtained from torpedo-stage SSEs on PGR-free MS medium after 3 months of culture. Complete plantlets could be readily excised and transferred to soil.

Key words: somaclonal variation, callus, somatic embryo, plant regeneration, oil palm

Introduction

Oil palm (*Elaeis guineensis* Jacq.) is a cross-pollinated crop grown in many tropical countries of Asia, Africa and South-Central America as a source of vegetable oil. Generally this plant propagates exclusively by seeds which are heterozygous in nature)Corley, 1982). The commercial seeds are sold as a

*Corresponding author: Te-chato, S; e-mail: stechato@yahoo.com

mixture of crosses since a single bunch produces only about 1,500 seeds (Rajanaidu *et al*., 1997). An oil palm tree is very important in terms of monetary value. In its productive life time of more than twenty years in the field, a palm produces about 150 kg (10 bunches x 15 kg) of fresh fruit bunches (FFB) per year and 3 tonnes of FFB over a twenty year period. The oil palm is also a crop species producing high quality oil, which can be obtained from the mesocarp of the fruit (palm oil) and the kernel of the nut (palm kernel oil). Palm oil is used mainly for cooking, preparation of margarine, shortening and also for non-food applications (soap, detergent, cosmetics, etc.) Gallo-Meagher *et al*., 2002; Chehmalee and Te-chato, 2007). For Thailand, the oil has been recently brought to production of biodisel. In the next five years (2011) the government have a policy to increase an area for oil palm planting to 10 million rai (1.6 million acre) (Thawaro and Te-chato, 2007). Accordingly a high yield plant are needed for fuel oil/biodisel production. Indeed, the large amount of oil produced in the oil palm fruit is unique biological characteristic of this palm species. Plant regeneration of oil palm through *in vitro* culture has been reported by several researchers (Te-Chato, 1998b). A reliable and efficient procedure for *in vitro* propagation of elite clone will increase yields in a significant way. Earlier studies from our laboratory were based on regeneration from leaf explants using dicamba (Te-chato *et al*., 2002). Primary callus induction from young leaf of hybrid tenera seedling has already been reported. Embryo culture has great potential for improving the efficiency of interspecific crosses (Zhang *et al*., 2003*)*. Culture of immature zygotic embryos (IZE) at various stages of development could lead to unique culture responses in comparison with mature zygotic embryos (MZE) (Teixeira *et al*., 1993). Dicamba has been reported to be an effective auxin for both shortening time period for callus induction and increasing a large number of somatic embryo and could be promoted callus more than one layer to produce callus (Te-chato *et al*., 2003). Dicamba at concentration of 1-2.5 mg/l stimulated proliferation rate of callus and embryogenic callus (Wang *et al*., 2006). Plantlets regenerated from culturing mature zygotic embryo has been reported by Te-chato (1998). However, percentage and numbers of new forming embryos were limited and germination frequency of those embryos quite low. From the success of both mature and immature zygotic embryo it is of great important in multiplication of hybrid oil palm from parents of elite dura and pisifera (DXP) crosses through tissue culture technique. The establishment of plant regeneration in oil palm by somatic embryogenesis is satisfactory, including ZE culture (Kanchanapoom and Domyoas, 1999). Various stages of ZE and genotypes of embryos were reported to be success by inducing somatic embryogenesis (Chehmalee and Te-chato, 2007). Dicamba has been reported to be an effective auxin for increasing

a large number of somatic embryos (SEs) (Te-chato *et al*., 2003). Regeneration of oil palm through secondary somatic embryos (SSEs) has also been reported using polyamines (Rajesh *et al*., 2003). However, percentage and number of new forming embryos were limited and germination of those embryos was not reported. Sorbitol was reported to induce SSE from haustorium embryo (HE) derived from culturing young leaf of mature oil palm subsequent to a high frequency of plantlet formation (Te-chato and Hilae, 2007).

Simple sequence repeat (SSR) marker provides one of the best PCR marker systems and the importance of SSR as a source of markers for plant genetics have been confirmed. With regards to oil palm, it was demonstrated that simple repetitive DNA was present in abundance in the oil palm (Cheah *et al*., 1995). It can be used to screen oil palm genomic DNA for di-, tri-, tetra- and pentanucleotide repeats and found them to be widely distributed in the oil palm genome. Initial attempts which construct SSR enriched genomic libraries were not successful, as less than 1% of the clones appeared to contain SSR motifs (Cheah and Wooi, 1993). Later, other researchers (Billotte *et al*., 2001) reported the successful construction of an oil palm SSR enriched genomic library, with 72% of the clones containing SSR motifs. They went on to demonstrate the applicability of the SSR markers in revealing the genetic relationships of populations of the genus *Elaeis* in accordance with their known geographical origins. They also demonstrated that the markers developed for one species, *E. guineensis*, were applicable in a second species, *E. oleifera*. Hence both micropropagation and molecular marker assisted selection is of great importance in the propagation of oil palm on the commercial scale. Before reaching that point, hybridity of the IZE should be verified at an early stage subsequent to mass propagation through verified IZE. In the present paper, we describe verification of genetic variation using calluses, somatic embryos and plantlets through SSR markers. So far, there have no reports on SSE formation in ZE culture. This report was to investigate the induction of SSE from IZE-derived HE subsequent to plantlet regeneration.

Materials and methods

Plant material

IZEs of 'Tenera' hybrid, derived from the cross number 7 and 16 at 3-4 months after pollination (MAP) were excised by the protocol described by Sakulrat and Te-chato (2008). Briefly, mesocarp was removed from fruit and cracked by hammer, then trimmed by pruning scissors to remove the excess kernel. IZE surrounded by kernel in cube of 5 mm×5 mm×8 mm were sterilized

in 70% alcohol for 30 sec, followed by 20% (w/v) sodium hypochlorite together with 1-2 drops of Tween-20 for further 20 min, followed by successive washing with sterile distilled water 3 times in laminar flow station. The embryos were aseptically removed from kernel and cultured on culture medium.

Establishment of culture

Sterilized IZE of 2 genotypes were inoculated in culture tubes containing 10 ml of MS medium supplemented with 2.5 mg/l dicamba for callus induction. The medium was solidified with 0.75% agar, adjusted pH to 5.7 with 0.1 N HCl before adding agar and autoclaved at 1.05 kg/cm^2, 121 ^{o}C for 15 min. The cultures were placed under light conditions at 25 µmol/m^{-2}/s^{-1} for 14 h photoperiod, at 27±1^{o}C and subcultured monthly intervals, on the same medium component for 3 months. Completely randomized design (CRD) with 5 replicates (each replicate consists of 10 embryos) was designed. The percentage of cultures producing callus was recorded and compared among those crosses.

Induction of somatic embryo (SE)

Calli derived from IZE were transferred to MS medium supplemented with 1 mg/l dicamba in order to induce somatic embryo. The medium was solidified with 0.75% agar, adjusted pH to 5.7 with 0.1 N HCl before adding agar and autoclaved at 1.05 kg/cm^2, 121^{o}C for 15 min. The cultures were placed under light conditions at 25 µmol/m^{-2}/s^{-1} for 14 h photoperiod, at 27±1oC and subcultured monthly intervals, on the same medium component for 3 months. Completely randomized design (CRD) with 5 replicates (each replicate consists of 10 embryos) was performed. The percentage of cultures producing somatic embryo was recorded and compared among those crosses.

Induction of secondary somatic embryo (SSE)

HEs of the two genotypes were carefully isolated and inoculated on hormone-free MS medium with 0.2 M sorbitol. The medium supplemented with 200 mg/l ascorbic acid and gelled with 0.75% agar-agar. There were 3 replicates, each containing 5 HEs/replicate. The cultures were maintained under the same conditions as described earlier. The number of SSE/HE was counted and percentage of SSE formation from cultured HE per tube was recorded after 1 month of culture.

Germination of SSE

SSE from haustorium-staged embryos at 2 months after culture on sorbitol containing medium were transferred to growth regulator-free MS medium supplemented with 3% sucrose solidified with 0.75% agar-agar. All cultures were carried out in bottle (60×110 mm containing 25 ml of medium) under the same conditions as described earlier. There were 3 replicates, each containing 10 SSEs/replicate. Germination percentage in terms of shoot, root and complete plantlet production was recorded after 3 months of culture.

Verification of somaclonal variation via SSR analysis

Callus, somatic embryo and leave from plantlet were collected (20 mg) and used for isolation of DNA according to the method described by Thawaro and Te-chato (2007). SSR analysis of genomic DNA was carried out using 8 microsatellite loci amplified in oil palm using 8 primers (EgCIR008, EgCIR0243, EgCIR0337, EgCIR0409, EgCIR0446, EgCIR0465, EgCIR0781 and EgCIR0905) (Table 1) as described by Billotte *et al*. (2001). Amplification of genomic DNA was done in a 10 µl mixture containing 2.5 mM MgCl2, 10× *Taq* buffer, 100 µM of each dNTP, 0.5 mM of each primer, 1.5 units of *Taq* polymerase and 20 ng of template DNA (Billotte *et al*., 2005). PCR amplifications were carried out on a thermo cycler (TC-XP-G, Japan) using the following program: denaturation at 95°C for 1 min, 35 cycles of 94°C for 30 s, 52°C for 60 s, 72°C for 120 s, and a final elongation step at 72°C for 8 min. An equal volume of loading buffer (98% formamide, 0.025% bromophenol blue, 0.05% xylene cyanol) was added to the amplified products, following denaturation at 94°C for 5 min. The products were separated on 6% (w/v) denaturing polyacrylamide gels and visualized with silver staining (Bassam *et al*., 1991).

Data analysis

Data were analysed by ANOVA. Mean among treatments was separated by Duncan's multiple range tests (DMRT) and Least significant difference (LSD) at the 0.01 or 0.05 level of probability. Where the F-test showed significant differences among mean.

Table 1. Primer used in SSR analysis of identified hybridity in the in the parents plant of *Elaeis quineensis* Jacq.

Primer name	Repeat motif	5'-3' Forward primer	5'-3' Reverse primer
EgCIR008	(GA)17	CGGAAAGAGGGAAGATG	ACCTTGATGATTGATGTGA
EgCIR0243	(GA)17	TGGAACTCCTATTTTACTGA	GCCTCGTAATCCTTGTCA
EgCIR0337	(GT)6 (GC)4	GTCTGCTAAAACATCAACTG	GAGGAGGAGGGGAACGATAA
EgCIR0409	(CCG)6	AGGGAATTGGAAGAAAAGAAAG	TCCTGAGCTGGGGTGGTC
EgCIR0446	(CCG)7	CCCCTTCGAATCCACTAT	CAAATCCGACAAATCAAC
EgCIR0465	(CCG)6	TCCCCCACGACCCATTC	GGCAGGAGAGGCAGCATTC
EgCIR0781	(GA)17	CCCCTCCCTACCACGTTCCA	TGTTTGCTGTTGCTCTTTGATTTC
EgCIR0905	(GT)14ctca (GA)11	CACCACATGAAGCAAGCAGT	CCTACCACAACCCCAGTCTC

Results

Effect of crosses on callus induction

Different genotypes gave the different responses on the percentage of cultures producing callus. Fresh IZE excised from seeds developed haustorium structure after culture for 4 weeks, then started to produce calluses. The callus initiation from IZEs was observed within 5 weeks of culture in callus induction medium. Cross number 7 gave the percentage of callus formation at 33.33 higher than cross number 16 (Fig. 1). However, cross number 16 gave the best result in percentage of speed of callus formation index (SCFI) at 24.77 (Fig. 1). Upon 1 month of subculture on fresh MS medium, types of calluses could be distinguished: compact nodular calluses were pale yellow (Fig. 2A) or yellow (Fig. 2B) in color and compact in appearance.

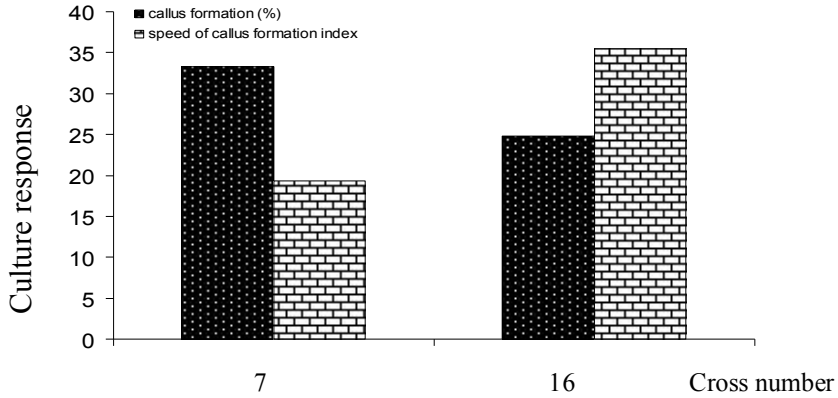

Fig. 1. Response of IZE of two crosses on MS medium supplemented with 3% sucrose, 200 mg/l ascorbic acid and 2.5 mg/l dicamba after 3 months of culture.

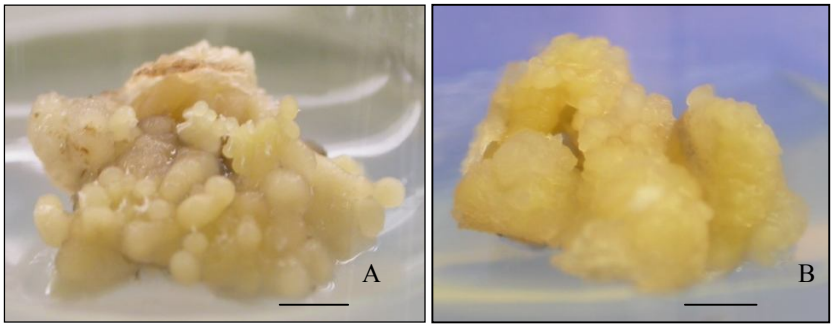

Fig. 2. Morphological characteristics of 3-month-old callus derived from culturing immature zygotic embryo culture of hybrid oil palm on MS supplemented with 3% sucrose, 200 mg/l ascorbic acid and 2.5 mg/l dicamba. (A) Compact nodular callus were pale yellow in color derived from cross number 7. (B) Compact nodular callus were yellow in color derived from cross number 16 (bar = 0.9 mm).

Induction of somatic embryo (SE)

The percentage of cultures producing embryogenic callus (EC) and number of somatic embryo per callus differed between the two crosses. Time consume for callus initiation from those embryos was 4-5 weeks of culture. After transferring the calli onto MS medium supplemented with 1 mg/l dicamba and culture under the same conditions for 2 months the higher SCFI was obtained from cross number 7. The higher rate of EC formation at 2.94 and number of SE/callus at 33.33 (Fig. 3, 4A) was obtained from cross number 7. In the present study, the result of this experiment indicated that cross number 7 had the hybrid vigor more than cross number 16. The morphological characteristics of embryogenic callus and somatic embryo among the two different crosses were shown in Fig. 4.

Induction of secondary somatic embryo (SSE)

SSE is a new cycle of somatic embryogenesis which could be induced directly from HE. For MS medium replacement sucrose with 0.2 M sorbitol in the present of 200 mg/l ascorbic acid gave better result in number of SSE formation at 18 SSE/HE than cross number 16 after 2 months of culture (Table 1). Sorbitol was proved to be a better stress agent in cross number 16 than cross number 7. In this experiment, it was found that SSE originated from the basal part of HE. Those SSEs were clustered with white opaque characteristic, consisted of torpedo-stage embryos (Fig. 5A) and mixes stage of embryos (Fig. 5B)

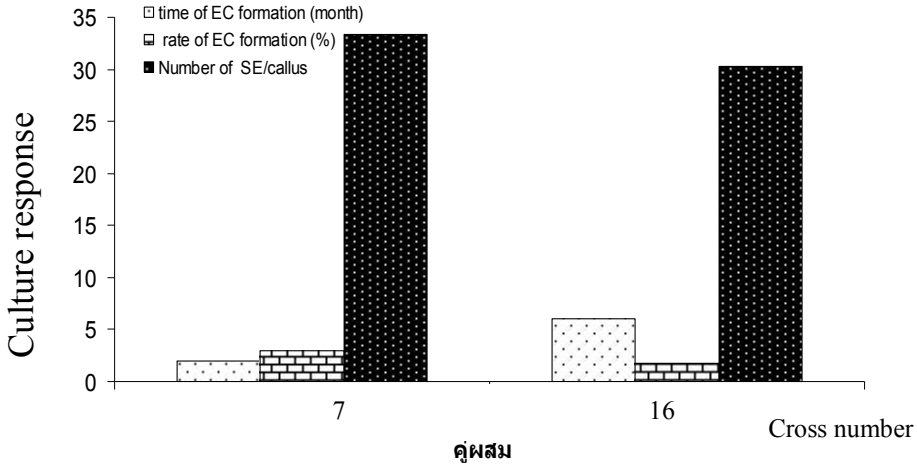

Fig. 3. Embryogenic callus and somatic embryo formation from 2 crosses on MS medium supplemented with 3% sucrose, 200 mg/l ascorbic acid and 1 mg/l dicamba after 3 months of culture.

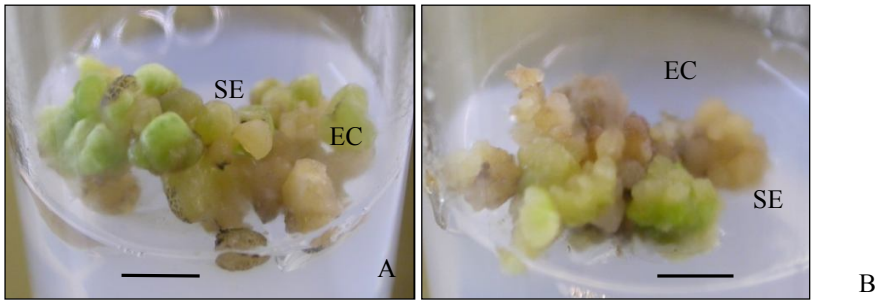

Fig. 4. Characteristics of EC and SE obtained from culturing IZEs of hybrid oil palm on MS medium supplemented with 3% sucrose, 200 mg/l ascorbic acid and 1 mg/l dicamba. (A) EC and SE derived from cross number 7. (B) EC and SE derived from cross number 16 (bar = 0.7 mm).

Table 2. Effect of period of culture on SSE formation on MS medium supplemented with 0.2 M sorbitol and 200 mg/l ascorbic acid.

Cross	**No. of SSE/HE**	
Time period (months)	**7**	**16**
1	5.6b [1/]	6.2b
2	13.8a	18a
F-test	**	**
C.V. (%)	15.8	14.01

** = Significant difference at P<0.01 level.
[1/] = Value followed by different letter are significantly different according to DMRT

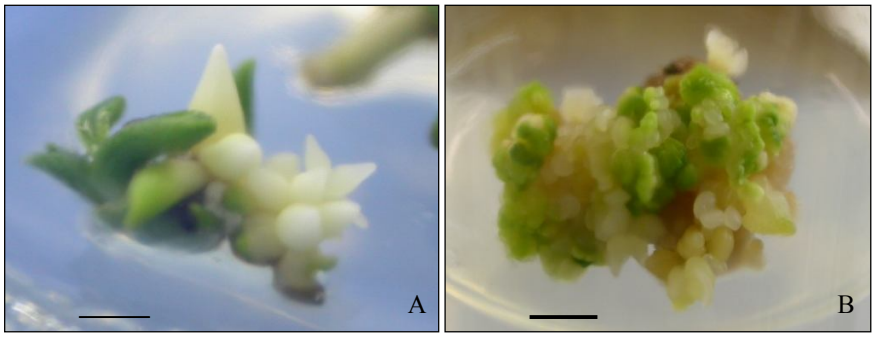

Fig. 5. Developmental of SSE after cultured on MS free medium supplemented with 3% sucrose, 200 mg/l ascorbic acid. (A) SSE at torpedo-stage embryos (bar = 0.3 mm). (B) SSE mixes stage embryos (bar = 0.5 mm).

Germination of secondary somatic embryo (SSE)

Increasing in time period for maintaining SSEs on MS supplemented with 0.2 M sorbitol before transferring to MS medium devoid plant growth regulators yielded the increment of germination of SSE by producing shoot, root and complete plantlet (Table 2). Complete plantlets regeneration (both shoot and root) formation at 0.8% was obtained from 3 months maintenance whereas 1 and 2 months failed to produce complete plantlets (Table 2). Cluster of torpedo-stage SSE were elongated (Fig. 6A,B) and individually isolated which further develop into shoot (Fig. 6C), root (Fig. 6D) and complete plantlets (Fig. 6E) on hormone-free MS medium with 3% sucrose for 3 months. This time period (2 months) of maintenance on sorbitol containing medium was enough to stress those SSE to be ready for germination.

Table 3. Effect of genotypes on germination of SSEs after culturing on hormone-free MS medium with 3% sucrose for 3 months.

Cross number	Germination of SSEs)month(	Average No. SSE producing shoot	Average No. SSE producing root	Average No. complete plantlet
7	1	0d [1/]	0	0
	2	4.07c	0	0
	3	16.8a	0.2	0.6
16	1	0d	0	0
	2	7.2b	0	0
	3	18.6a	0.2	0.8
F-test		**	-	-
C.V. (%)		24.59		

** = Significant difference at P<0.01 level, = Data not shown, [1/] = Value followed by different letter are significantly different according to DMRT.

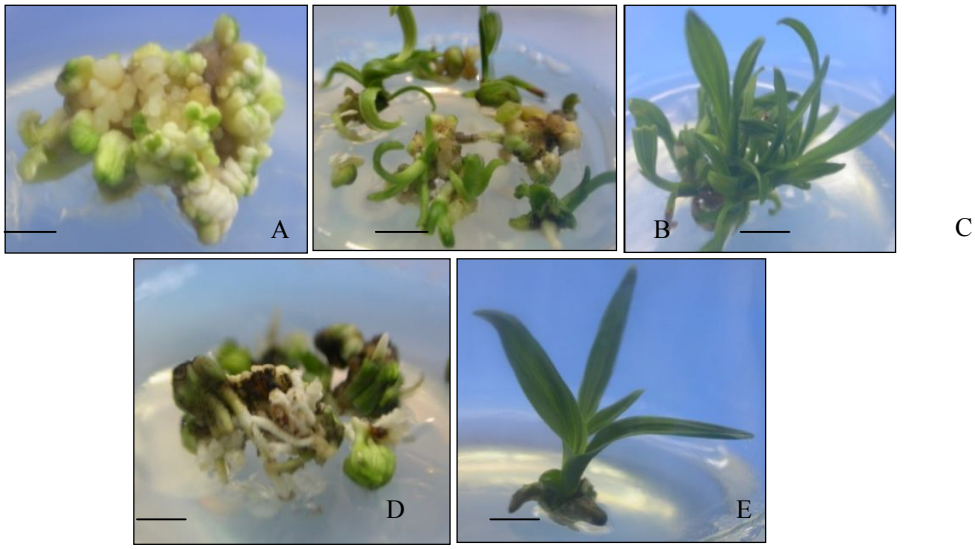

Fig. 6. Development of SSE from HE (A) on 0.2 M sorbitol containing MS medium for 1 month of culture (bar: 3.25 mm) subsequent to germination of SSE (B) on hormone-free MS medium (bar: 6 mm) for further 3 months. (C) Multiple shoot. (D) Root without shoot. (E) Complete plantlet.

Verification of somaclonal variation via SSR analysis

Genomic DNA from the callus, somatic embryo and leave of plantlet of oil palm were amplified using 8 primers. PCR products were observed in each of the profiles, however, primers EgCIR008 provided a clear DNA pattern and could be amplified and provided polymorphic patterns of DNA from the both parents. This primer showed the greatest capacity for distinguishing polymorphic fragments in callus and somatic embryo cultured from the parent. The results of the DNA pattern from the cross 7 and 16 showed a specific fragment and could be used to distinguish between dura and pisifera palms. All callus and somatic embryo of hybrids showed the DNA patterns between the two parents and more additive bands according to SSR analysis. Eighteen samples of callus from cross number 7 were heterozygous with primer EgCIR008 showing both a male parent-specific band at 550 bps, and a female parent-specific band at 600 bps (Fig. 7A). Cross number 16 showing both a male parent-specific band at 590 and 610 bps, and a female parent-specific band at 580 and 600 bps (Fig. 7B). SSR fingerprint indicating uniformity of callus, somatic embryo and plantlet among the different lines. The DNA profile remains unchanged between lines of both crosses (Fig. 8A, B).

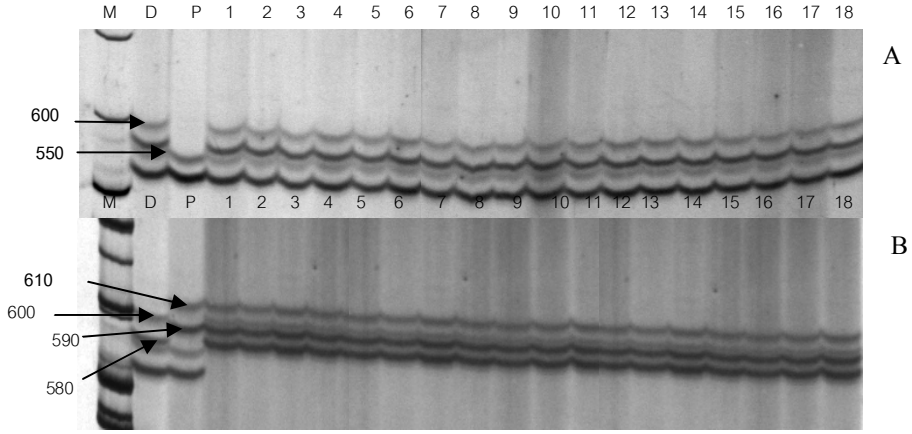

Fig. 7. DNA patterns in callus of hybrids and parents of genotype obtained with SSR primers EgCIR008. Amplification of products were compared on the basis of molecular size. Lane M: standard DNA (100 bp plus DNA ladder). Lanes P and D: fragments from parents. Lanes 1–18: fragments from hybrids. (A) Cross number 7. (B) Cross number 16.

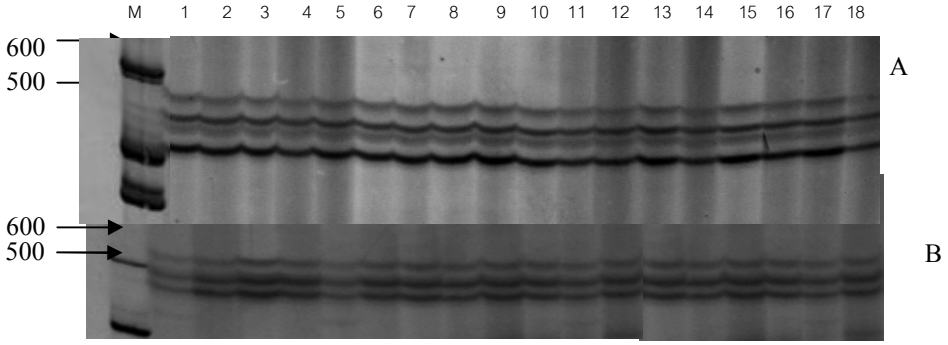

Fig. 8. DNA fingerprint of 18 somatic embryos derived from IZEs indicating uniformity among the different lines of callus. Lane 1-8 were fragment from individual somatic embryo and lane 9-18 from regenerated plantlets. The DNA profile remains unchanged between the two cross**.** (A) Cross number 7. (B) Cross number 16.

Discussion

Dicamba was effective in inducing callus from embryo culturing of wheat (*Triticum aestivum* L.) (Mendoza and Kaeppler, 2002). A similar result was also found in immature embryo cultures of winter wheat (Carman *et al*., 1988) and spring wheat cultivars (Hunsinguer and Schauz, 1987). Dicamba is a promising auxin which has been reported to be effective in promoting direct and indirect embryogenic callus induction from cultured mature zygotic embryo and young leaf of oil palm (Te-chato, 1998). For culturing of IZE, MS medium

supplemented with 2.5 mg/l dicamba gave the highest NC formation from cross number 7. Genotypes of the selected explants may have influenced upon the type of responsive callus like the report of Sarasan *et al.* (2005). In addition, medium containing dicamba was reported to induce nodular structure from both epidermal cells and vascular tissues while 2,4-D induced this only from the epidermis (Te-chato *et al*., 2003). For proliferation of embryogenic callus and formation of SE, MS medium supplemented with 1 mg/l dicamba gave an optimum proliferation of embryogenic callus and formation of SE. Dicamba was found to be the best auxin for mass propagation in vitro of both seedling and mature oil palm [5]. The differences in the embryogenic callus response of the genotypes might be depended on genetic make up of each parents and growing conditions of the donor plants. Similar results were observed by Rines and McCoy (1981) in oats Duncan *et al*. (1985) in maize and Berthouly and Michaux-Ferriere (1996) in coffee. Crossings or genotypes play important role in somatic embryogenesis (Steinmacher *et al*., 2007). Different varieties result in the different response on SE formation and plantlets development (Karun *et al*., 2004). Decrease in concentration of dicamba stimulated proliferation rate of EC and also promoted a large number of embryoid formation (Wang *et al*., 2006). Moreover, in our previous study, the larger seeds consisted of larger size of ZE of all crosses gave the higher percentage of germination and callus formation (Te-chato and Hilae, 2007). Some authors reported that low concentration of dicamba promoted somatic embryogenesis from immature inflorescence (Steinmacher *et al*., 2007).

SSE is a new cycle of somatic embryogenesis which could be induced directly from HE. Full-strength MS medium supplemented with 0.2 M sorbitol produced significantly higher percentage and number of SSEs (Te-chato and Hilae, 2007). Polyamines were also reported to induce SSE from culturing ZE of oil palm, however, percentage and number of new forming embryos still low (Rajesh *et al*., 2003). So, sorbitol was proved to be a better stress agent than polyamine in promoting SSE both percentage and number. In this experiment, it was found that SSE originated from the basal part of HE. Those SSEs were clustered with white opaque characteristic consisted of torpedo-stage embryos. Promchan and Te-chato (2007) reported that SSE arose directly from epidermal layer at the basal part of HE. This time period (2 months) of maintenance on sorbitol containing medium was enough to stress those SSE to be ready for germination. This process might relate to the hydrolysis of food reserves in the endosperm and the mobilization of nutrients required for embryos germination like the report of Sarasan *et al*. (2005). Those complete plantlets could be readily excised and transferred to soil.

For SSR markers, the SSR pattern among the cross number 7 and 16 with primer EgCIR008 showed the presence of both male and female fragments of DNA. These results were similar to those reporting the use of SSR markers for testing seed genetic purity of commercial hybrid tomato cultivars (Liu *et al*., 2007). Although the somaclonal variation phenomenon that gives rise to abnormal palms is a common occurrence in oil palm (James *et al*., 2002), records indicate that the "rogue" palm in this case was producing normal inflorescences and fruit bunch. From the present study, it is clear that somaclones obtained from our protocol were uniform according to the SSR markers. Using SSR, various authors have reported the absence of genetic variation in trees like *Picea mariana* (Isabel *et al*., 1993) and *Pinus thunburghii* (Goto *et al*., 1998). It also demonstrates that genetic integrity of micropropagated plants should invariably be confirmed before transfer of hardened plants to field.

In conclusion, different genotypes gave the different response on the percentage of cultures producing callus, number of SE/callus, SSE/HE and germination of SSE. The present study successfully describes *in vitro* plant regeneration protocol of IZE via somatic embryogenesis. The higher rate of EC formation (2.94) and number of SE/callus (33.33) was obtained from cross number 7. MS medium replacement sucrose with 0.2 M sorbitol in the present of 200 mg/l ascorbic acid gave better result in number of SSE formation at 18 SSE/HE than cross number 16 after 2 months of culture. Plantlet conversion rate at 0.8 % from SSEs was obtained from 3 month maintenance of HE on sorbitol containing medium subsequent to culture on PGR-free MS medium. Primer EgCIR008 of SSR provided clear DNA patterns and could be used for verification the hybridity and somaclonal variation of both cross.

Acknowledgments

The authors are grateful to the Agricultural Research Development Agency, the Faculty of Natural Resources and the Graduate School of Prince of Songkla University and the Oil Palm Agronomical Research Center (OPARC) of Southern Thailand for financial support.

References

Bassam, B.J., Anolles, G.C., Gresshoff, P.M. (1991). Fast and sensitive silver staining of DNA in polyacrylamide gels. Analytical Biochemistry 196: 80–83.

Berthouly, M. and Michaux-Ferriere, N.M. (1996). High frequency somatic embryogenesis in *Coffea canephora*: Induction conditions and histological evolution. Plant Cell Tissue and Organ Culture 44: 169–176.

Billotte, N., Marseillac, N., Risterucci, A.M., Adon, B., Brottier, P., Baurens, F.C., Singh, R., Herrran, A., Asmady, H., Billot, C., Amblard, P., Durand-Gasselin, T., Courtois, B.,

Asmono, D., Cheah, S.C., Rohde, W., Ritter, E. and Charrier, A. (2005). Microsatellite-based high density linkage map in oil palm (*Elaeis guineensis* Jacq.). Theoretical and Applied Genetics 110, 754–765.

Billotte, N., Risterucci, A.M., Barcellos, E., Noyer, J.L., Amblard, P. and Baurens, F.C. (2001). Development, characterisation and across-taxa utility of oil palm (*Elaeis guineensis* Jacq.) microsatellite markers. Genome 44: 1–14.

Carman, J.G., Jefferson, N.E. and Campbell, W.F. (1988) Induction of embryogenic *Triticum aestivum* L. calli. I. Quantification of genotype and culture medium effects. Plant Cell Tissue and Organ Culture 12: 83–95.

Cheah, S.C. and Wooi, K.C. (1993). Application of molecular techniques in oil palm tissue culture. In: Proceedings of the International Society for Oil Palm Breeders Symposium, Kuala Lumpur, pp 163–170.

Cheah, S.C., Sambanthamurthi, R., Akmar, S.N., Abrizah, A., Manaf, O., Umi, M.A.A., Salamah, R., Parveez, G.K.A. (1995). Towards genetic engineering of oil palm (*Elaeis guineensis* Jacq.). In: Kader JC, Mazliak P (eds) Plant Lipid Metabolism, Kluwer, Dordrecht, pp 570–572.

Chehmalee, S. and Te-chato, S. (2007). Genotypes, physiological ages of zygotic embryos and auxin as affect on germination and callus formation of oil palm. International Conference on Integration of Science & Technology for Sustainable Development, Bangkok, Thailand. 26 – 27 April 2007, 166-169.

Corley, R.H.V. (1982). Clonal planting material for the oil palm industry. The Planter 58: 515-528.

Duncan, D.R., Williams, M.E., Zehr, B.E. and Widholm, J.M.. (1985). The production of callus capable of plant regeneration from immature embryos of numerous *Zea mays* genotypes. Planta 165: 322–332.

Gallo-Meagher, M. and Green, J. (2002). Somatic embryogenesis and plant regeneration from immature embryos of saw palmetto, an important landscape and medicinal plant. Plant Cell Tissue and Organ Culture 68: 253–256.

Goto, S., Thakur, R.C., Ishii, K. (1998). Determination of genetic stability in long term micropropagated shoots of Pinus thunburghii Parl. using RAPD markers. Plant Cell Reports 18: 193–197.

Hunsinguer, H., Schauz, K. (1987). The frequency of dicamba on somatic embryogenesis and frequency of plant regeneration from cultured immature embryos of wheat (Triticum aestivum L.). Plant Breeding 98: 119–123.

Isabel, N., Tremblay, L., Michaud, M., Tremblay, F.M. and Bousquet, J. (1993). RAPDs as an aid to evaluate the genetic integrity of somatic embryogenesis derived population of Picea mariana (Mill.) B.S.P. Theoretical and Applied Genetics 86: 81–87.

James, W.T., Fabienne, M., Frederique, R., Angelique, B., Rajinder, S., Suan, C.C., Caroline, H., Alain, R. and Yves, D. (2002). Characterization of a defensin gene expressed in oil palm inflorescences: induction during tissue culture and possible association with epigenetic somaclonal variation events. Journal of Experimental Botany 53: 1387-1396

Kanchanapoom, K. and Domyoas, P. (1999). The origin and development of embryoids in oil palm (*Elaeis quineensis* Jacq.) embryo culture. ScienceAsia 25: 195-202.

Karun, A., Siril, E.A., Radha, E. and Parthasarathy, V.A. (2004). Somatic embryogenesis and plantlet regeneration from leaf and inflorescence explants of arecanut (*Areca catechu* L.). Current Science 86: 1623-1628.

Liu, L.W., Wang, Y., Gong, Y.Q., Zhao, T.M., Liu, G., Li, X.Y., Yu, F.M. (2007). Assessment of genetic purity of tomato (*Lycopersicon esculentum* L.) hybrid using molecular markers. Scientia Horticulturae 115: 7–12.

Mendoza, M.G., Kaeppler, H.F. (2002). Auxin and sugar effects on callus induction and plant regeneration frequencies from mature embryos of wheat (Triticum aestivum L.). In Vitro Cellular & Developmental Biology - Plant 38: 39–45.

Promchan, T. and Te-chato, S. (2007). Size of haustorium embryo affecting secondary somatic embryo formation of oil palm and its origin. International Conference on Integration of Science and Technology for Sustainable Development, Bangkok, Thailand. 26-27 April 2007. pp. 22-25.

Rajanaidu, N., Rohani, O. and Jalani, B.S. (1997). Oil palm clones: current status and prospects for commercial production. The Planter 73: 163-184.

Rajesh, M.K., Radha, E., Karun, A. and Parthasarathy, V.A. (2003). Plant regeneration from embryo-derived callus of oil palm–the effect of exogenous polyamines. Plant Cell, Tissue and Organ Culture 75: 41–47.

Rines, H.W. and McCoy, T.J. (1981). Tissue culture initiation and plant regeneration in hexaploid species of oats. Crop Science 21: 837–842.

Sarasan, V., Ramsay, M.M. and Roberts, A.V. (2005). Rescue of endangered palms by *in vitro* methods: the case of 'bottle palm'. *In* Protocol for Somatic Embryogenesis in Wood Plants. Vol. 77, pp. 267-274. The Netherlands: Springer.

Steinmacher, D.A., Clement, C.R. and Guerra, M.P. (2007). Somatic embryogenesis from immature peach palm inflorescence explants: towards development of an efficient protocol. Plant Cell, Tissue and Organ Culture 89: 15–22.

Te-chato, S. (1998). Callus induction from cultured zygotic embryo of oil palm subsequent to plantlet regeneration. Songklanakarin Journal of Science and Technology 20: 1-6.

Te-chato, S. and Hilae, A. (2007). High-frequency plant regeneration through secondary somatic embryogenesis in oil palm (*Elaeis guineensis* Jacq. *var*. tenera). Journal of Agricultural Technology 3: 345-357.

Te-chato, S., Hilae, A. and Yeedum, I. (2002). Improve callus induction and embryogenic callus formation from cultured young leaves of oil palm seeding. Journal of Agricultural Sciences 35: 407-413.

Te-chato, S., Hilae, A. and Yeedum, I. (2003). Histological study on oil palm of somatic embryos development as affected by sources of leaf explants and auxin. Journal of Agricultural Science 36: 243-250.

Te-chato, S., Hilae, A. and Yeedum, I. (2003). Histological study on oil palm of somatic embryos development as affected by sources of leaf explants and auxin. Journal of Agricultural Science 36: 243-250.

Teixeira, J.B., Sondahl, M.R, Nakamura, T. and Kirby, E.G. (1993). Somatic embryogenesis from immature zygotic embryos of oil palm. Plant Cell, Tissue and Organ Culture 34: 227-233.

Thawaro, S. and Te-chato, S. (2007). Auxins as an effect on types of callus formation from maturezygotic embryo culture of hybrid oil palms. International Conference on Integration of Science & Technology for Sustainable Development, Bangkok, Thailand. 26 – 27 April 2007, 166-169.

Wang, H.C., Chen, J.T. and Chang, W.C. (2006). Somatic embryogenesis and plant regeneration from leaf, root and stem-derived callus cultures of *Areca catechu*. *Journal* of *Plant Biology* 50: 279-282.

Zhang, G.Q., Zhou, W.J, Gu, H.H., Song, W.J. and Momoh, E.J.J. (2003). Journal Agronomy and Crop Science 189: 347-350.

(Received 29 June 2010; accepted 7 March 2011)