EFFECT OF GENOTYPES OF OIL PALM ON CALLUS, EMBRYOGENIC CALLUS AND SOMATIC EMBRYO FORMATION

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Abstract

Immature zygotic embryos (IZE) of 16 crosses were excised and cultured on Murashige and Skoog (MS) medium supplemented with 2.5 mg/l dicamba (3,6-dichloro-o-anisic acid) to initiate callus. The cultures were placed under light conditions at 14 h photoperiod, 27±1°c for 3 months. The result revealed that the highest percentage of callus formation (33.33) was obtained from cross number 7. Time consume for callus initiated from these embryos was 4-5 weeks of culture. After that the calli were transferred to culture on MS medium supplemented with 1 mg/l dicamba and culture under the same conditions. The highest speed of embryogenic callus formation was obtained from cross number 1 and 9 after 1 month of culture. The highest percentage of embryogenic callus (7.69) was obtained from cross number 1 and average number of somatic embryo formation (33.33 embryos) was obtained from cross number 7. Cross number 7 and 16 gave the highest response on somatic embryo formation and multiplication to haustorium stage. So they represent good quality materials for study of secondary somatic embryo (SSE) formation and plant regeneration.

Keywords: genotype, callus, embryogenic callus, somatic embryo, oil palm

Introduction

Oil palm is one of the most economically important crops in the world. The DxP denomination is common to all planters and agronomists in the oil palm plantations. For the layman, D stands for Dura whereas P stands for Pisifera. The fruits produced through pollinating the Pisifera pollen onto the D female flowers are called DxP. The palms raise from DxP seeds are called Tenera (commercial oil palm). The Tenera oil palm scenery is commonly observed in the south of Thailand. However, the fresh fruit bunch production capability and oil extraction rate among the Tenera's depend on their genetics (ASD costa rica, 2012). Cultivation of oil palm has expanded tremendously in recent years such that it is now second only to soybean as a major source of the world supply of oils and fats (Wahid *et al.*, 2004). Interest in palm oil as a biofuel could eventually cause constraints on worldwide supply of edible palm oil and increase the pressure for higher yield and/or cultivatable areas (Biofuel, 2007). The oil palm has only a single growing point, and does not produce suckers like some other palm species, so clones cannot be produced by the common techniques such as cutting, grafting or layering (Corley and Tinker, 2003). So it is possible to enhance efficiency for propagation through somatic embryogenesis, especially *in vitro* culture through zygotic embryo (ZE) culture and also embryo explants are convenient because fruits are

readily available, have a high degree of physiological uniformity, and can be shipped long distances. The establishment of plant regeneration in oil palm by somatic embryogenesis is satisfactory, including ZE culture (Te-chato, 1998a; Kanchanapoom and Domyoas, 1999). Various stages of ZE and genotypes of embryos were reported to be success by inducing somatic embryogenesis (Teixeira *et al*., 1993 and 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). Plantlets regenerated from culturing mature zygotic embryo has been reported by Te-chato (1998b). However, percentage and numbers of new forming embryos were limited and germination frequency of those embryos quite low. From ZE-derived HE subsequent to plantlet regeneration. 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 though tissue culture technique. Plantlets obtained from this procedure should be an elite hybrid suit for propagation in the field. So, commercial scale propagation of superior genotypes is possible. Vigor of the seeds is generally assessed by germination test and demonstrated as germination speed index (GSI). Normally, vigor seed or zygotic embryo also has a huge silence power for germination and other activity include callus formation. The previous paper report about callus formation (Sanputawong and Te-chato, 2008). Therefore, we want to refer to previous and continuous to the new works. In this paper, callus, embryogenic callus and somatic embryo induction from IZE of hybrid from 16 crosses of oil palm were described.

Methodology

Plant material

Immature oil palm fruits at 3 months after fertilization (MAF) of the 16 crosses from DxP were kindly provided by Khun Anek Lim. IZEs of 'Tenera' hybrid 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.

Effect of crosses on callus formation

Sterilized IZE of 16 genotypes were inoculated in culture tubes containing 10 ml of MS medium supplemented with 2.5 mg/l dicamba and 200 mg/l ascorbic acid 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/cm2, 121^{o}C for 15 min. The test tubes were wrapped with parafilm. The cultures were grown in a temperature controlled chamber at 27±1 °C under an 14-h photoperiod, unless stated differently. Photosynthetic photon flux density of 25 μmol $m^{-2}s^{-1}$ was provided by "white" fluorescent tubes. The culture was regularly transferred to fresh MS medium monthly interval on the same medium component for 3 months. Each experiment was performed with 5 replicates. Each replicate consisted of 5 test tubes (25×150 mm containing 10 ml of medium). The percentage of cultures producing callus and type of callus were recorded and compared among those crosses.

Effect of crosses on embryogenic callus and somatic embryo formation

IZE derived primary callus from Expt. I was transferred to fresh MS medium supplemented with 1 mg/l dicamba, 200 mg/l ascorbic acid, 3% sucrose in order to induction of embryogenic callus

and somatic embryo induction. The test tubes were wrapped with parafilm. The culture was maintained under the same conditions as described in callus induction and subcultured monthly intervals. Each experiment was performed with 5 replicates. Each replicate consisted of 10 test tubes (25×150 mm containing 10 ml of medium). The percentage of cultures producing embryogenic callus, time of embryogenic callus formation and somatic embryo were recorded and compared among those crosses.

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

Results

Effect of crosses on callus induction Different genotypes gave the different response on the percentage of cultures producing callus and type of callus. Fresh IZE excised from seeds (Fig. 1A) were developed haustorium structure (Fig. 1B) after culture for 4 weeks, then it started to produce calluses. The callus initiation from IZEs was observed within 5 weeks of culture in callus induction medium. However, some of IZEs did not respond after culture for 4 weeks (Fig. 1C) and 3 months (Fig. 1D). Upon 1 month of subculture on MS medium, four types of calluses could be distinguished: compact (Fig. 2A, B), friable (Fig. 2C), nodular (Fig. 3D) and root-like calluses (Fig. 2E). Compact calluses were yellow or pale yellow in color and compact in appearance. Friable calluses were yellow, translucent and succulent. The compact nodular calluses were yellow and consisted of small nodules. In case of root-like calluses, they were elongative, white and soft. Cross number 7 gave the highest percentage of callus (33.33) among other cross after 3 months of culture (Fig. 3), followed by cross number 14 and 16, respectively. Cross number 16 gave the best result in percentage of callus formation at 24.77 (Fig. 3). As indicated in Fig. 3, the response of the genotypes on callus formation had a wide range from 7% to 33%. Two crosses; (cross number 7 and 14) were classified as high capacity in their callus formation, significant different to other crosses. Genotypes of the selected explants may have influenced upon the type of responsive callus. In this present study it is clear evident that genotype play role in type of callus. The comparison of morphological characteristics of callus among 16 different crosses induced from immature zygotic embryos of oil palm showed that the compact callus was obtained from cross number 5 (17.647), friable callus from cross number 14 (44), root like callus from cross number 3 (37.143) and nodular callus from cross number 7 (52.941) gave the best percentage each type of callus (Fig. 4). The result of these experiments are summarized in Fig. 3-4.

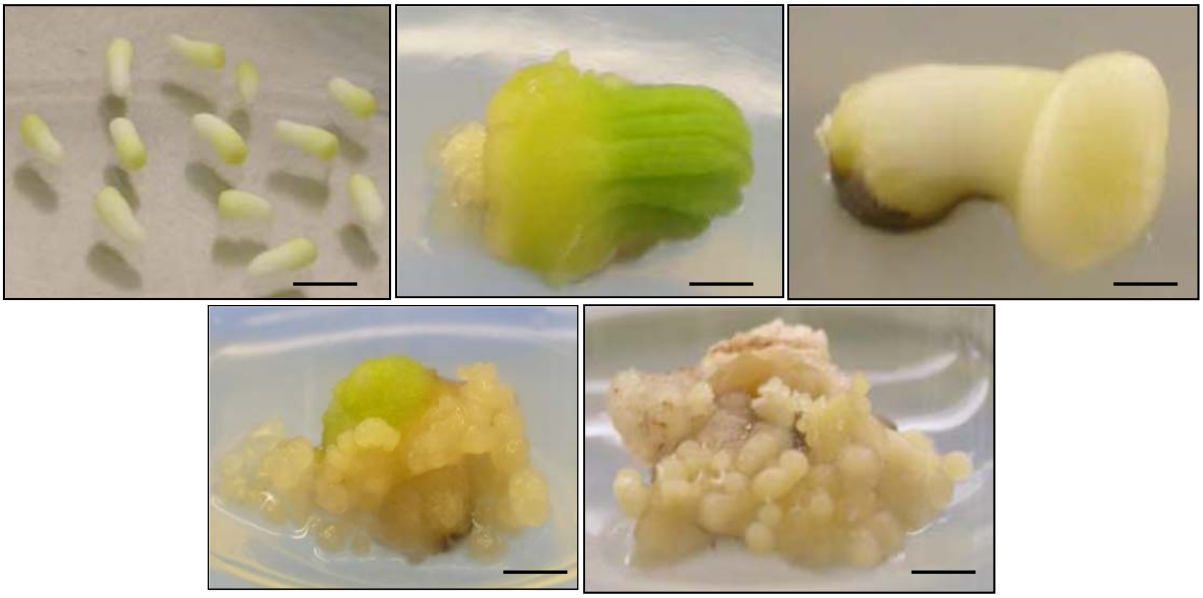

Figure 1 Morphological characteristics of 3-month-old callus derived from culturing IZE of hybrid oil palm on MS supplemented with 3% sucrose, 200 mg/l ascorbic acid and 2.5 mg/l dicamba. (A) Fresh isolation of IZE (bar = 0.3 mm). (B) Haustorium forming IZE (bar = 0.5 mm). (C) Dormant IZE (bar = 0.3 mm). (D) Primary callus (bar = 0.5 mm). (E) Nodular callus (bar = 0.5 mm).

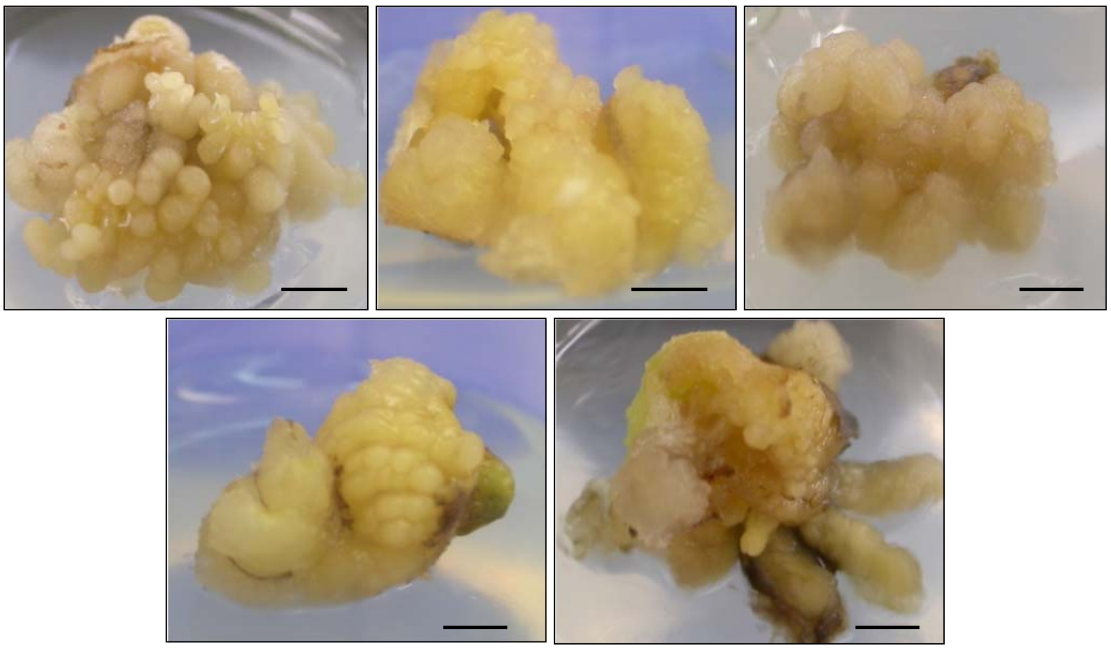

Figure 2 Morphological characteristics of types of 3-month-old callus derived from culturing immature zygotic embryo culture of hybrid oil palm on MS supplemented with 3% sucrose, 200 mg/l Ascorbic acid and 2.5 mg/l dicamba. (A,B) Compact nodular callus (bar = 0.9 mm). (C) Friable nodular callus (bar = 0.5 mm). (D) Nodular callus (bar = 0.5 mm). (E) root-like callus (bar = 0.7 mm).

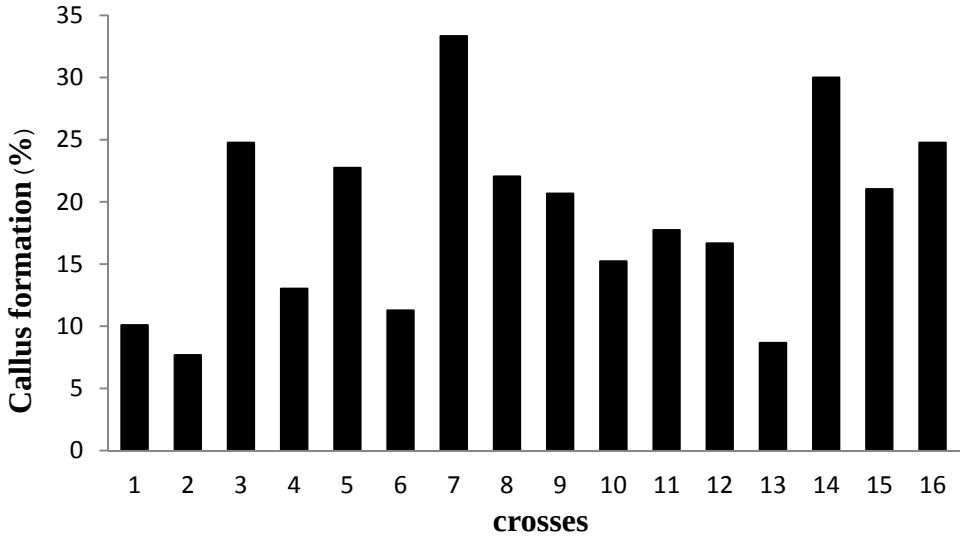

Figure 3 Response of IZE of 16 crosses on MS medium supplemented with 3% sucrose, 200 mg/l Ascorbic acid and 2.5 mg/l dicamba after 3 months of culture.

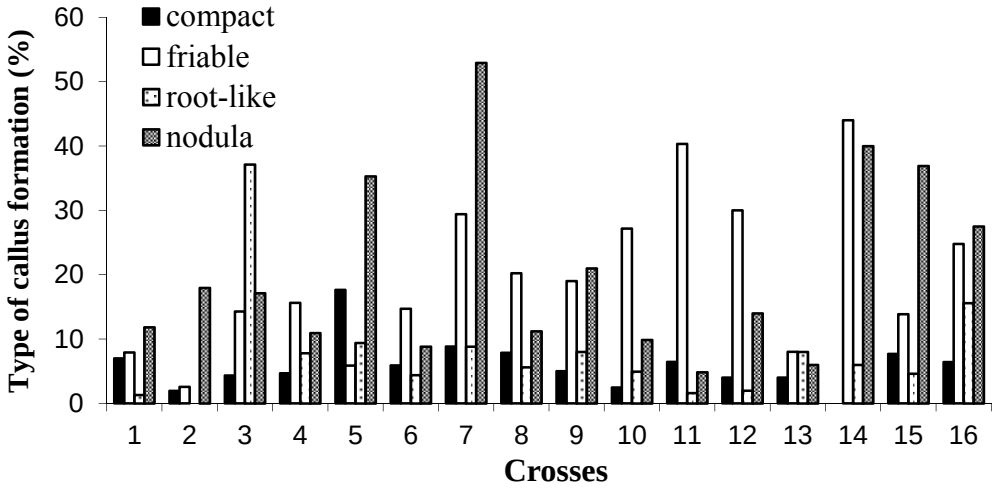

Figure 4 Response of IZE of 16 crosses on MS medium supplemented with 3% sucrose, 200 mg/l Ascorbic acid and 2.5 mg/l dicamba after 3 months of culture.

Effect of crosses on embryogenic callus and somatic embryo induction

The percentage of cultures producing embryogenic callus (EC), number of embryogenic callus per explant and speed of EC formation varied from cross to cross. Cross number 1 gave the highest percentage of embryogenic callus formation (7.69) and the embryogenic callus response of the genotypes varied from 1.54% to 7.69% (Fig. 5A). Four crosses (cross number 7, 11, 14 and 16) gave the high frequency of embryogenic callus formation. The best result in speed of EC formation at 1 month was obtained from cross number 9 and 11 (Fig. 5B), followed by 5 and 7, respectively. 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. For the somatic embryo formation, the cross number 7 gave the highest number of SE per callus (33.33) after 3 months of culture (Fig. 5C). From this present study it is suggest that cross number 9 and 11 have the hybrid vigor more than another crosses. EC/SE formation occur on the base and the face of callus after 3 months of culture (Fig. 6.). The reason could be due to the good combining ability of gene between the two parents. The morphological characteristics of embryogenic callus and somatic embryo among the 10

different crosses were shown in Fig. 7. Cross number 7 and 16 gave the highest response on somatic embryo formation and multiplication to haustorium stage. So, they represent good quality materials for study of secondary somatic embryo (SSE) formation and plant regeneration.

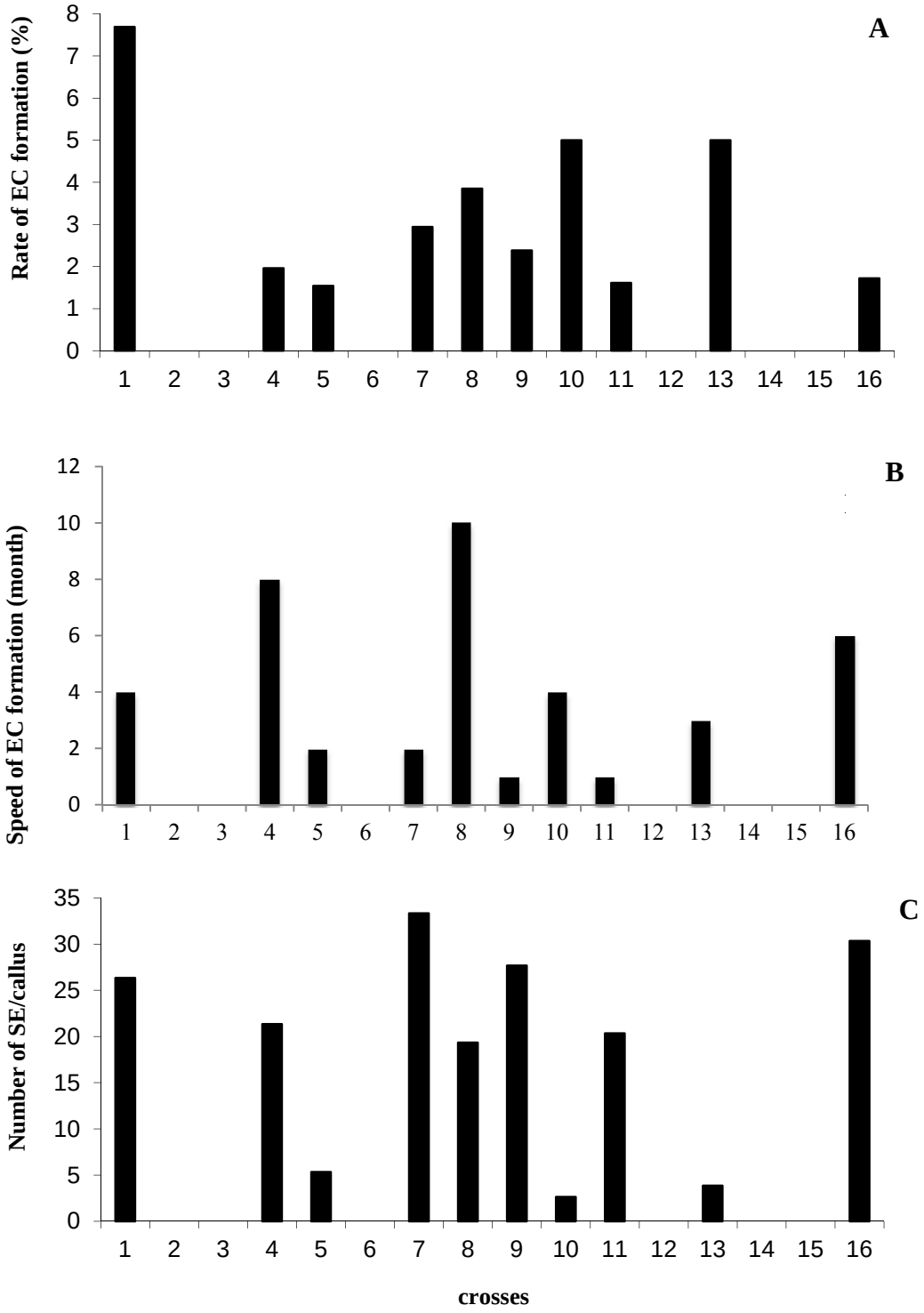

Figure 5 Embryogenic callus formation from 16 crosses on MS medium supplemented with 3% sucrose, 200 mg/l ascorbic acid and 1 mg/l dicamba after 3 months of culture. Effect of cross combination on number of EC/callus formation (A) time of EC formation (B) and number of SE/callus formation (C).

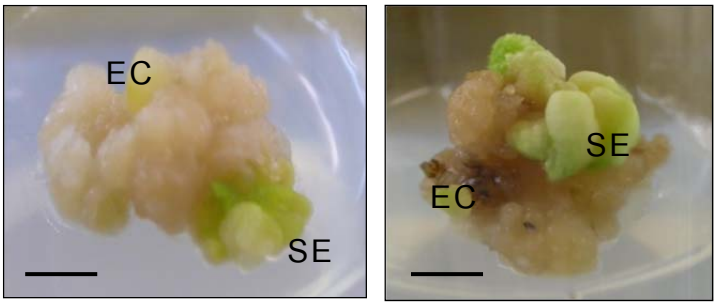

Figure 6 EC/SE formation occur on the base (left) and the face (right) of callus (bar = 1.5 cm.) after culture on MS supplemented with 1 mg/l dicamba, 200 mg/l ascorbic acid and 3% sucrose after 3 months of culture.

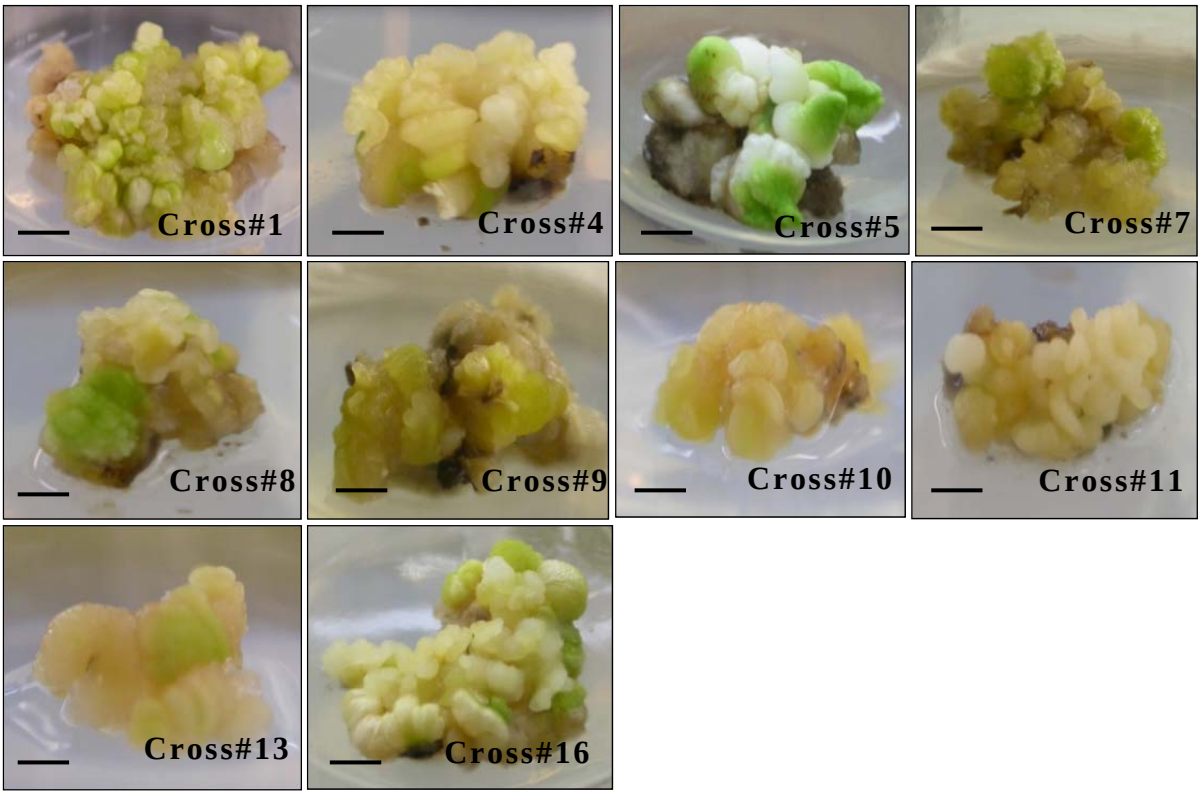

Figure 7 Morphological characteristics of EC and SE obtained from culturing IZE of hybrid oil palm on MS medium supplemented with 1 mg/l dicamba, 200 mg/l ascorbic acid and 3% sucrose (bar = 1 cm).

Discussions and Conclusion

Different genotypes gave the different response on the percentage of cultures producing callus, type of callus, embryogenic callus, speed of embryogenic callus and somatic embryo formation. Dicamba was effective in inducing callus from embryo culturing of wheat (*Triticum aestivum* L.) (Mendoza and Kaeppler, 2002 and Thawaro and Te-chato, 2007). 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, 1998b). For culturing of IZE, MS medium supplemented with 2.5 mg/l dicamba gave the highest nodula callus formation from cross number 7. Two crosses; (cross number 7 and 14) were classified as high capacity in their callus formation, significant different to other crosses. Similar results were also reported by Sanchez-Romero (2005) in Avocado, Sairam *et al.*(2003) in soybean, El-Bakry (2002) in tomato and Diana (2002) in coffee. 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). Dicamba was found to be the best auxin for mass propagation in vitro of both seedling and mature oil palm. Decrease in concentration of dicamba stimulated proliferation rate of EC and also promoted a large number of embryoid formation (Wang *et al*., 2006). Some authors reported that low concentration of dicamba promoted somatic embryogenesis from immature inflorescence (Steinmacher *et al*., 2007). 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. The highest speed of embryogenic callus formation was obtained from cross number 1 and 9 after 1 month of culture. The highest percentage of embryogenic callus was obtained from cross number 1 and average number of somatic embryo formation was obtained from cross number 7. Crossings or genotypes play important role in somatic embryogenesis (Steinmacher *et al*., 2007). The differences in the embryogenic callus, SE formation and plantlets development (Karun *et al*., 2004) 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 Karun *et al*. (2004) in oil palm Rines and McCoy (1981) in oats Duncan *et al*. (1985) in maize and Berthouly and Michaux-Ferriere (1996) in coffee. 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). In the present study, cross number 7 and 16 gave the highest response on somatic embryo formation and multiplication to haustorium stage. So, it represents good quality material for study of SSE formation and plant regeneration.

Acknowledgments

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