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Effect of genotypes of oil palm as indicator for speed of callus and embryogenic callus formation

Sakulrat Sanputawong and Sompong Te-chato*

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

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Among sixteen crosses of immature zygotic embryo was investigated for their effects on callus and embryogenic callus formation. Immature zygotic embryos were excised and cultured on Murashige and Skoog (MS) medium supplemented with 2.5 mg/l dicamba (3, 6-dichloro-o-anisic acid). The cultures were placed under light conditions at 14 h photoperiod, 27±1 ^{o}C for 3 months. The results revealed that the highest percentage of callus formation (33.33) was obtained from cross number 7 and percentage of embryogenic callus formation (18) was obtained from cross number 14. The highest number of embryogenic callus formation per explant (15.795) was obtained from cross number 16 after 3 months of culture. Callus initiated from these embryos within 4-5 weeks and classified into 4 types; compact, friable, nodular and root-like structure. The highest increment in callus size (1.17 cm) was obtained from cross number 16. In case of the speed index of callus and embryogenic callus formation, cross 16 gave the highest of both parameters at 35.5 and 20.17, respectively.

Key words: genotypes, oil palm, callus, embryogenic callus

Introduction

The 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. It is propagated exclusively by seeds which are heterozygous in nature (Corley, 1982). The commercial seeds are sold as a 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

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

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 and Green, 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 (1998a). 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 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. In this paper, callus and embryogenic callus induction from IZE of hybrid from 16 crosses of oil palm were described.

Materials and methods

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. IZE of those hybrid (cross number 1 to 16) were excised by the following procedure. 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 5×5×8 mm^3 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 induction

Sterilized IZE of 16 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^oC for 15 min. The cultures were placed under light conditions at 1,300 lux illumination for 14 h photoperiod, at 27±1^oC and subcultured monthly intervals, on the same medium component for 3 months. Completely randomized design (CRD) with 4 replicates (each replicate consists of 10 embryos) was designed. The percentage of cultures producing callus, size of callus, type of callus and speed of callus formation index (SCFI) were recorded and compared among those crosses.

Effect of crosses on embryogenic callus induction

IZE derived callus from Expt. I was transferred to MS medium supplemented with 2.5 mg/l dicamba in order to induction of embryogenic 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^oC for 15 min. The cultures were placed under light conditions at 1,300 lux illumination for 14 h photoperiod, at 27±1^oC and subcultured monthly intervals, on the same medium component for 3 months. Completely randomized design (CRD) with 4 replicates (each replicate consists of 10 embryos) was performed. The percentage of cultures producing embryogenic

callus and speed of embryogenic callus formation index (SECFI) were recorded and compared among those crosses.

Results and discussion

Effect of crosses on callus induction

Different genotypes gave the different response on the percentage of cultures producing callus, size of callus, type of callus and SCFI. 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). Upon 1 month of subculture on MS medium, four types of calluses could be distinguished: compact (Fig. 1D, E), friable (Fig. 1F), nodular (Fig. 1G) and root-like calluses (Fig. 1H). 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. 2A), followed by cross number 14 and 16, respectively. Cross number 16 gave the best result in percentage of callus formation at 24.77 (Fig. 2A). As indicated in Fig. 2A, 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. 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. For SCFI it was showed that cross number 16 gave the highest result at 35.5 (Fig. 2A), followed by cross number 9 (30.67) and cross number 5 (26.5), respectively. Even though cross number 14 and 11 gave the best result for SCFI at 19.5 and 14.5 in the first time, but cross number 16 gave the best result finally. It is indicated that cross number 16 had the hybrid vigor more than another crosses (Fig. 2A). Genotypes of the selected explants may have influenced upon the type of responsive callus like the report of Sarasan *et al.* (2005). 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. 2B). The result of these experiments are summarized in Fig. 3.

More over cross combination played important role on average size of callus after 3 months of culture. Cross number 7 gave the largest size of callus after culture for 1 and 2 months (Fig. 2C), however, cross number 16 gave the bigger size (1.67 cm) of callus after 3 months of culture, followed by cross number 7 (1.42 cm.) and 14 (1.05 cm.), respectively. Cross number 7 gave the best result on average size of callus within 3 months of culture (Fig. 2C).

Effect of crosses on embryogenic callus induction

The percentage of cultures producing embryogenic callus, number of embryogenic callus per explant and SECFI varied from cross to cross. Cross number 14 gave the highest percentage of embryogenic callus formation (18) whereas the cross number 16 gave the highest number of nodule per callus (15.79 nodule/EC) after 3 months of culture (Fig. 3A). Cross number 16 gave the best result for both percentage of embryogenic callus (16.52) and number of nodule per callus (15.79 nodule/EC) (Fig. 3A). Embryogenic callus response of the genotypes varied from 0.4% to 18% (Fig. 3A). Four crosses (cross number 7, 11, 14 and 16) gave the high frequency of embryogenic callus formation. 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-Ferrière (1996) in coffee. The best result in SECFI at 20.17 was obtained from cross number 16 (Fig. 3B), followed by 14 and 11, respectively. From this present study it is suggest that cross number 16 have the hybrid vigor more than another crosses (Fig. 3B). The reason could be due to the good combining ability of gene between the two parents.

Conclusion

Different genotypes gave the different response on the percentage of cultures producing callus, size of callus, type of callus, SCFI, embryogenic callus, number of embryogenic callus per explant and SECFI. In the present study, the result of our experiment indicated that cross number 16 had the hybrid vigor more than another crosses and also the genotypes of the selected of explants may have influenced upon the type of callus. MS medium

supplemented with 2.50 mg/l dicamba gave the highest SCFI and SECFI at 35.5 and 20.167, respectively, significant difference to others crosses.

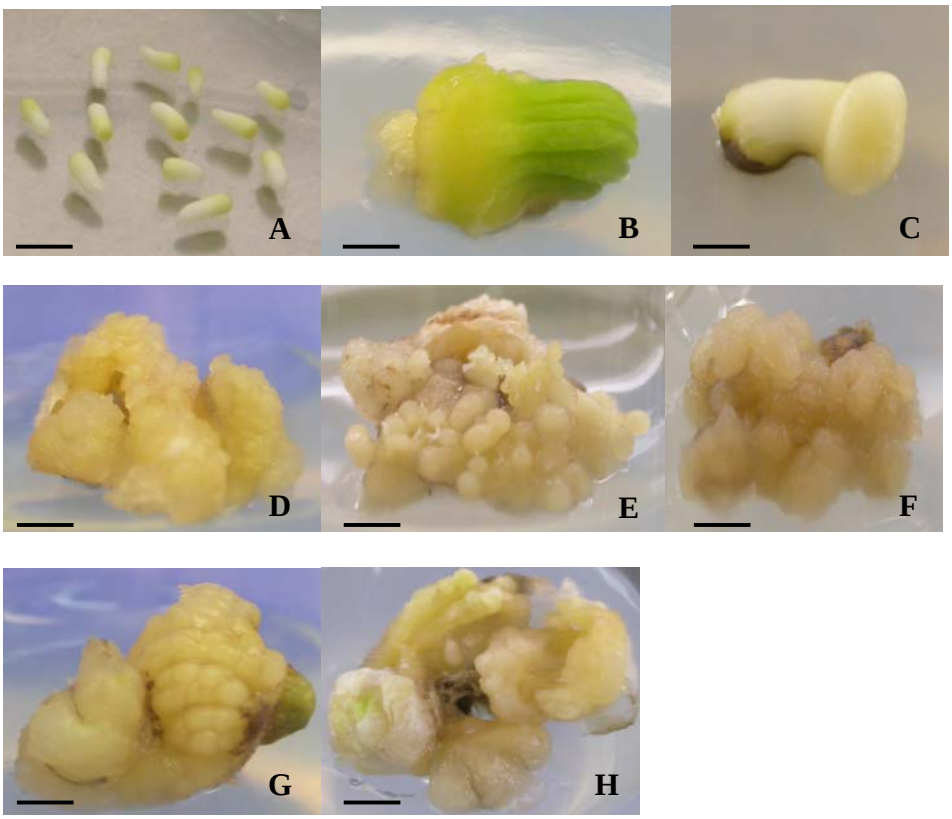

Fig. 1. Morphological characteristics of 3-month-old callus derived from culturing immature zygotic embryo culture of hybrid oil palm on MS supplemented with 3% sucrose, 200 Ascorbic acid and 2.5 mg/l dicamba. (A) Fresh culture of IZE (bar = 0.3 mm). (B) Haustorium forming IZE (bar = 0.5 mm). (C) Dormant IZE (bar = 0.3 mm). (D,E) Compact nodular callus (bar = 0.9 mm). (F) Friable nodular callus (bar = 0.5 mm). (G) Nodular callus (bar = 0.5 mm). (H) root-like callus (bar = 0.7 mm).

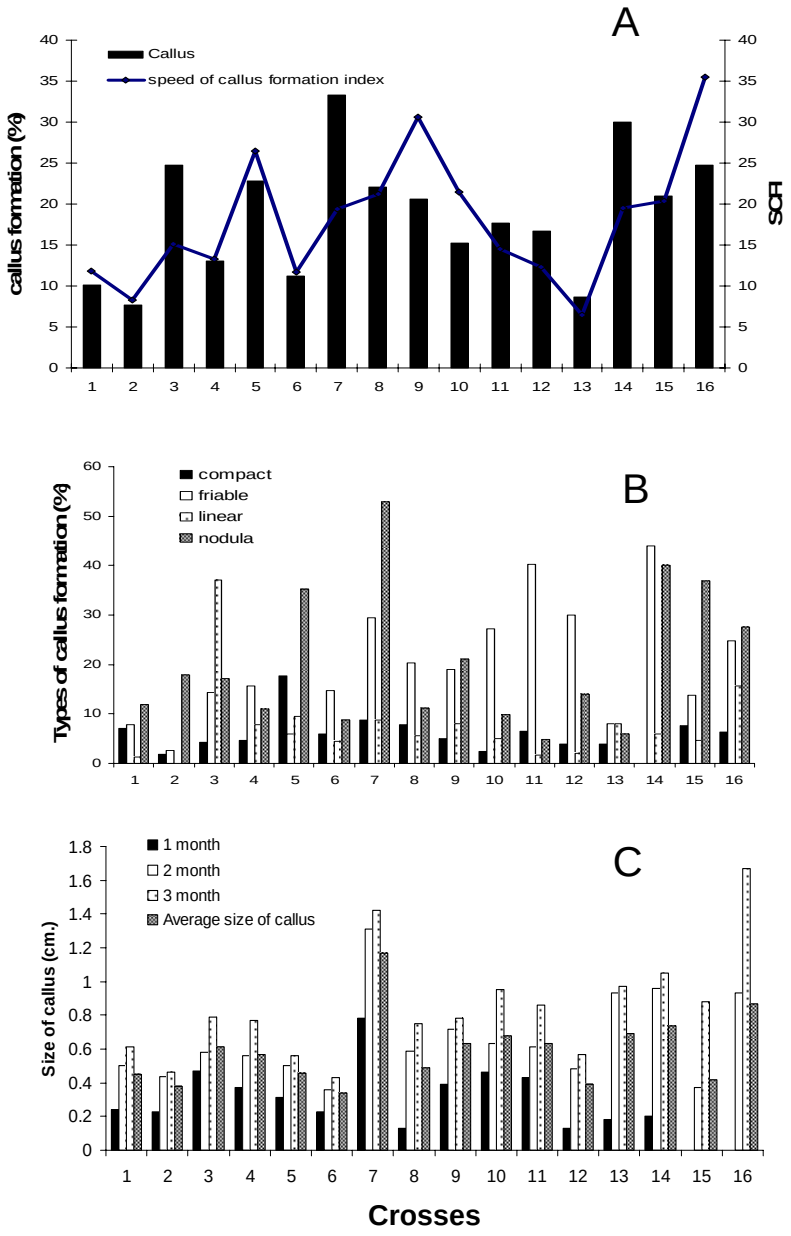

Fig. 2. Response of IZE of 16 crosses on MS medium supplemented with 3% sucrose, 200 mg/l Ascorbic acid and 2.5 mg/l dicamba after 3 months of culture. Effect of cross combination on callus formation and SCFI (A), types of calluses (B) and average size of callus (C).

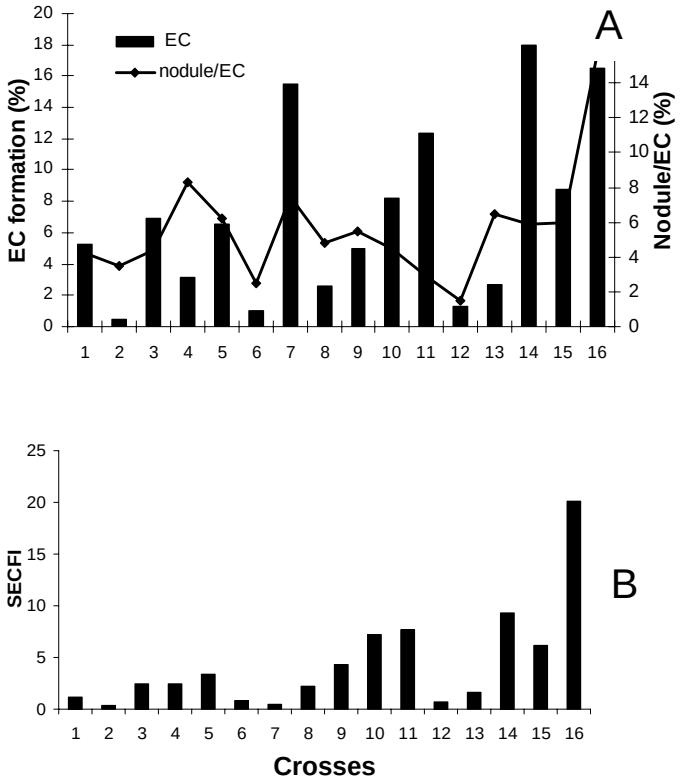

Fig. 3. Response of embryogenic callus of 16 crosses on MS medium supplemented with 3% sucrose, 200 Ascorbic acid and 2.5 mg/l dicamba after 3 months of culture. Effect of cross combination on embryogenic callus and #nodule/EC (A) and SECFI (B).

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References

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.

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.

Chen, L, Nan, L., Zhen, H., Pin, W., Chuanbei, L. and Mingshan, S. (2004). Molecular assay of novel pasturage, sugarcane-grass 94-42, using RAPD markers. Genetic Resources and Crop Evolution 51: 351–354.

Cordeiro, G.M., Taylor, G.O. and Henry, R.J. (2000). Characterisation of microsatellite markers from sugarcane (*Saccharum* sp.), a highly polyploid species. Plant Science 155: 161–168.

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

Diana, M.M., Aponte, M.E., Cortina, H. and Moreno, G. (2002). The effect of genotype and explant age on somatic embryogenesis of coffee. Plant Cell Tissue and Organ Culture 71: 117–123.

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.

Duval, Y., Engezmann, F. and Durand-Gasselin, T. (1995). Somatic embryogenesis in oil palm (*Elaeis guineensis* Jacq.). *In* Biotechnology in Agriculture and Forestry (ed. Y.P.S. Bajaj) Vol. 30, pp. 335-352, Heidelberg :Springer-Verlag.

El-Bakry, A.A. (2002). Effect of genotype, growth regulators, carbon source, and pH on shoot induction and plant regeneration in tomato. In Vitro Cell. Dev. Biol. Plant. 38: 501–507.

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.

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.

Malaysia's Sustainable Palm Oil. (2007). Palm oil facts [Online]. Available: http://www.Soyatech .com/Palm_Oil_Facts.htm. Access on August 19, 2007.

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.

Sairam, R.V, Franklin G., Hassel, R., Smith, B., Meeker, K., Kashikar, N., Parani, M., Abed, D. A., Ismail, S., Berry, K. and Goldman, S.L. (2003). A study on the effect of genotypes, plant growth regulators and sugars in promoting plant regeneration via organogenesis from soybean cotyledonary nodal callus. Plant Cell, Tissue and Organ Culture 75: 79–85.

Sanchez-Romero, C., Marquez-Martin, B. and Pliego-Alfaro, F. (2005). Somatic and zygotic embryogenesis in avocado. Plant Cell Monographs 2: 271-284.

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.

Te-chato, S. (1998a). Callus induction from cultured zygotic embryo of oil palm subsequent to plantlet regeneration. J. 20: 1-6.

Te-chato, S. (1998b). Fertile plant from young leaves-derived somatic embryos of oil palm. . 20: 7-13.

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.

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*. Biol Plant 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.

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