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SOMATIC EMBRYOGENESIS ON CALLI DERIVED FROM LEAF AND INFLORESCENCE TISSUES OF OIL PALM (*Elaeis guineensis* Jacq.)

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Abstract —This study describes a procedure for induction and proliferation of somatic embryos of calli induced from leaf and inflorescence tissues of oil palm (*Elaeis guineensis* Jacq.). Calli with embryogenic competence were cultured on modified Murashige and Skoog medium supplemented with 0.5 g/L glutamine, 0.1 g/L adenine sulphate plus 2,4-D (2.5, 5.0 mg/L) and NAA (2.5, 5.0 mg/L). Other additives were sucrose at 30 g/L and agar at 8 g/L. Media was pH 5.8, before autoclaving at 121^{o}C, 1Kg cm^{-2} pressure for 15-20 min. Cultures were incubated at 25 ± 2ºC under continuous light at 200 lux. All the treatments produced normal somatic embryos which are whitish with smooth surface detaching from the callus. Somatic embryos formed readily from leaf-derived callus than that of inflorescence at relatively lower concentrations of 2.5 mg/L 2,4-D. However, the PGR-free medium was more efficient for embryo multiplication with an almost indefinite proliferation of mature somatic embryos.

***Keywords* — callus, *Elaeis guineensis*, PGRs, somatic embryos**

INTRODUCTION

The oil palm (*Elaeis guineensis* Jacq.) belongs to the family Arecaceae (Corley and Tinker, 2003). It is an important agricultural commodity and the most efficient oil-bearing crop in the world (Jayanthi et al., 2015). The oil palm industry accounts for a significant sector of the Nigerian economy. It is developed to benefit the people more. Also, to increase gross domestic products and national income. Harnessing the benefits that tissue culture provides therefore becomes imperative. Tissue culture has become a veritable tool for producing superior and uniform planting materials throughout the year without any seasonal constraints.

In this study, the most important determinant step of in vitro systems which is somatic embryogenesis is considered. Williams and Masheshwaran (1986), described somatic embryogenesis as the process by which somatic cells develop into differentiated plants without fusion of gametes. It is the hub of in vitro multiplication as it is advantageous for mass propagation, genetic improvement programs, production of synthetic seeds (Hartmann et al., 2002), and provides a source of regenerable protoplasts (Chang and Wong, 1994). Somatic embryos can be cryopreserved which makes it possible to establish gene banks. Furthermore, somatic embryogenesis is an attractive system to study the morphology, biochemistry, genetic and molecular mechanisms of embryo development.

MATERIALS AND METHODS

Plant materials, media and culture conditions

Callus was induced from non-chlorophyllous leaf and immature inflorescence tissues obtained from NIFOR elite oil palm trees Tenera fruit form (D x P) (Iserhienrhien et al., 2012). Accordingly, explants were sterilized using standard procedures in 70% ethanol and 1.0% sodium hypochlorite solution, followed by gentle agitation for 10 minutes and rinsed with three changes of distilled sterile water. Explants were then immersed in 100 mg/L of citric, ascorbic and L-cysteine for 30 minutes with three changes of distilled sterile water. Explants were sliced into thin portions and implanted on culture medium for callus induction. For embryogenesis study, modified Murashige and Skoog (1962) salts at half strength constituted the basal media, plus 0.5 g/L glutamine, 0.1 g/L adenine sulphate. Sucrose at 30 g/L was used as carbon source. Agar at 8 g/L was used to solidify the medium without inclusion of activated charcoal. Auxin supplementations were 2,4-D (2.5, 5.0 mg/L) and NAA (2.5, 5.0 mg/L). Culture media were adjusted to pH 5.8 before autoclaving at 121^{o}C at 1Kg cm^{-2} pressure for 15-20 min. Implanted cultures were incubated in growth rooms at 25 ± 2^{o}C and maintained in continuous light at 200 lux during the period of study.

Approximate 40g masses of callus derived from leaf and inflorescence tissues were respectively pooled and transferred to basal media supplemented with NAA (2.5, 5.0 mg/l), 2, 4-D (2.5, 5.0 mg/l), and plant growth regulator free (PGR-free) as treatments. The duration for the onset of somatic embryo among treatments was monitored. Somatic embryos produced were estimated by counting under a magnifying glass and mean values were recorded every 4 weeks for 16 weeks.

Where applicable, mean and standard error were calculated from data obtained. Data were subjected to analysis of variance using SPSS program version 17. The differences among treatment means were determined by Duncan's multiple range tests at 0.05 % level of significance (Duncan, 1955).

RESULTS AND DISCUSSION

Eight (8) weeks after callus was transferred to embryogenesis medium, it had grown and multiplied to about 3 times its original size, producing friable embryogenic tissues (Figure 1). Some did not exhibit embryogenic potentials as no further changes in terms of growth were observed. These remained soft, jelly-like and translucent. The transfer of embryogenic tissues to the tested variables led to somatic embryo production.

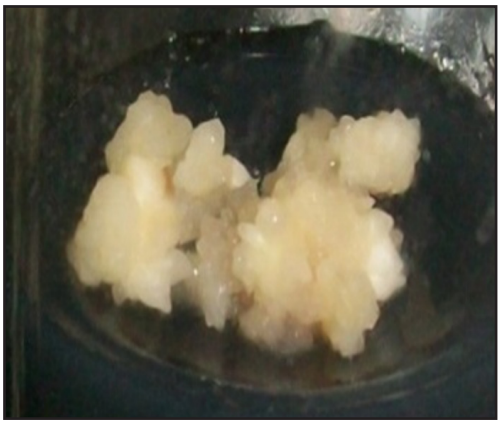

Figure 1. Embryogenic callus

Somatic embryo formation was marked by the production of white to yellowish spherical or nodular structures which later easily separated from the callus mass (Figure 2).

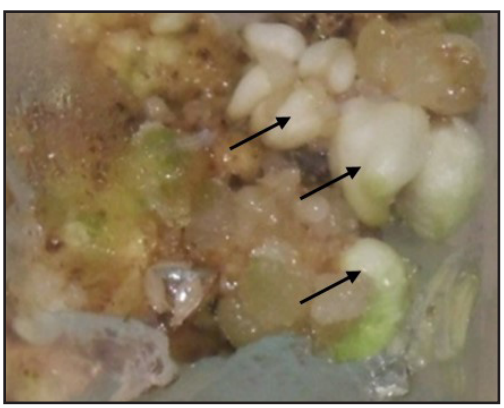

Figure 2. Developing somatic embryos

Some of these embryos proliferated indefinitely in culture producing secondary somatic embryos. Other embryos multiplied slowly producing poly-embryos which were bigger and for the most part greenish, heart-shaped and cotyledonary at maturity, with spontaneous germination (Figure 3).

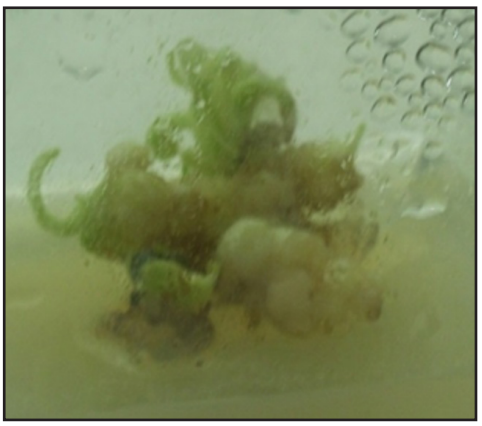

Figure 3. Germinating somatic embryos

Leaf and inflorescence derived calli grown on different treatments showed varying time with onset of production of somatic embryos as shown in Table 1. For leaf induced callus, 2.5 mg/L 2,4-D treatment showed first signs of somatic embryo production four weeks after, while 2.5 mg/L NAA showed first signs of somatic embryos at 6 weeks. For inflorescence induced callus somatic embryos were first observed in 2.5 mg/L 2,4-D at 8 weeks and 2.5 mg/L NAA at 10 weeks. For PGR-free medium, somatic embryos were observed after 6 and 10 weeks both for leaf and inflorescence-derived calli respectively.

Table 1. Initiation of somatic embryogenesis on leaf and inflorescence-derived calli under different treatments.

Treatment		Time (Wks.)	
Hormone	Conc. (mg/l)	Leaf	Inflorescence
PGR-f	0.0	6	10
2,4-D	2.5	4	8
	5.0	6	10
NAA	2.5	6	10
	5.0	8	12

At the 4th and 8th weeks, the PGR-free medium recorded with the least mean number of 10.33±1.45 and 20.33±2.60 somatic embryos respectively compared to 2,4-D and NAA supplemented media (Figures 4 & 5). These were significantly different at 5% level of probability. From the 12th up to the 16th week, a change in trend was observed. The rate at which somatic embryos were produced in the PGR-free medium was faster than 2,4-D and NAA supplemented media. At the 16th week, the PGR-free medium recorded the highest mean number of 86.67±17.64 somatic embryos while NAA 2.5 mg/l and 2,4-D 2.5 mg/l had 60±11.55 and 40±5.77 somatic embryos respectively. These results also showed significant differences among treatment means at 5% level of probability. For both 2,4-D and NAA supplemented media, it was observed that the higher the concentration, the less numbers of somatic embryos were produced with time.

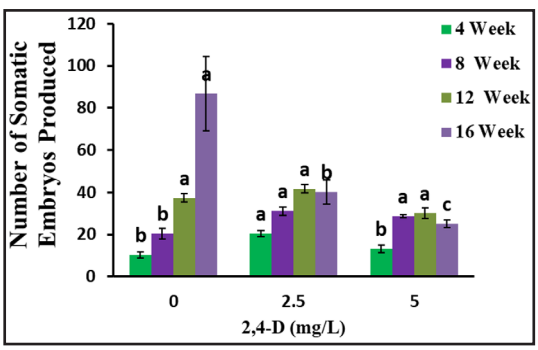

Figure 4. Number of somatic embryos produced by callus derived from leaf explants after different incubation periods in 2, 4-D supplemented medium.

(The values expressed are means ± SE. Bars with same colour bearing similar alphabet are not significantly different using Duncan's Multiple Range test.)

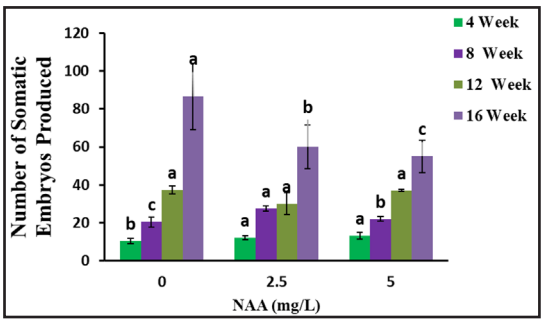

Figure 5. Number of somatic embryos produced by callus derived from leaf explants after different incubation periods in NAA supplemented medium.

(The values expressed are means ± SE. Bars with same colour bearing similar alphabet are not significantly different using Duncan's Multiple Range test.)

DISCUSSION

The success of any in vitro mass propagation depends on the rate of somatic embryos formation. Somatic embryos are formed when somatic cells, under conditions suitable for induction, generate embryogenic cells, which go through a series of morphological and biochemical changes that result in the production of a bipolar structure without vascular connection with the original tissue (Solis Ramos et al., 2012). Somatic embryos have the capability under the favorable nutrient and hormonal combination to multiply indefinitely leading subsequently to plantlet formation.

Specific tissues in various species have been found to either have a capacity (competence) for somatic embryogenesis in culture systems or can be induced to develop competency in culture by specific treatment to the medium (Hartmann et al., 2002). Various factors had been found to affect the induction of somatic embryogenesis in oil palm and other palm species. These include, but not limited to, the type and level of phyto-hormones, genotype of starting material, origin and developmental stage of

the explants used, media composition and physical culture conditions such as light and temperature (Purohit, 2008, Gaj, 2004).

The reduction of PGR in the medium from the initial concentrations enhanced the growth and proliferation of callus leading to embryoids production. This was consistent with (Paranjothy and Rohani, 1982, Nwankwo and Krikorian, 1983, Sogeke, 1996, Muniran et al., 2008). In this study, normal somatic embryos were produced among the various treatments. The somatic embryos were characterized by a bipolar structure with a root and shoot axis and well-defined cotyledons at maturity as described by Sahijram and Bahadur (2015).

In this study, there was a difference in time for somatic embryo induction from callus induced from leaf and inflorescence explants and among the PGR treatments used. The induction of somatic embryos from callus obtained from inflorescence tissues took relatively longer time than that from leaf tissues. This is most likely due to inherent factors rather than external influence as both leaf and inflorescence calli were subjected to the same conditions.

Also, comparing time of somatic embryo induction and concentrations of PGRs applied in cultures, 2,4-D induced embryos formed earlier than NAA in both leaf and inflorescence tissues. In addition, the concentration at which SE were first induced was lower than for NAA suggesting that 2,4-D maybe more efficient in SE induction than NAA in both leaf and inflorescence explants.

Relatively lower concentrations of the hormones tested were found to be better for somatic embryogenesis induction. It is interesting to note that beyond induction of somatic embryogenesis, the PGR-free medium was more efficient for embryos multiplication as it led to an almost indefinite proliferation of mature somatic embryos.

It was observed in this study that beyond the 12th week in the embryogenesis phase, the medium without PGR began to produce more embryos and at a faster rate than the other media supplemented with PGRs. It seems therefore that though auxin is required to trigger somatic embryogenesis, its role changes thereafter and embryos at a point begin to synthesize their own hormone, possibly, by an alternative pathway (Zimmermann, 1993). This phenomenon also agrees with Dodeman (1997) description of ‘’embryogenic calli’’ to be cells which have been able to achieve a change from somatic cell to a stage where it can develop into a somatic embryo without the requirement of further external stimuli.

CONCLUSION

Somatic embryos induced from leaf and inflorescence tissues of oil palm are important material for oil palm clonal propagation. From our findings, it took a shorter time for calli induced from leaf to produced somatic embryos than from inflorescence. Also, 2,4-D treatments induced somatic embryos earlier in both leaf and inflorescence derived calli than NAA treatment. Therefore, the success of this study will provide a framework for further studies.

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