Journal of Agricultural Technology 2008, V.4(2): 157-163

Effects of cytokinin types and concentrations on growth and development of cell suspension culture of oil palm

Sompong Te-chato[1*], Asalan Hilae[2] and Komgrit In-peuy[3]

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

Te-chato, S, Hilae, A. and In-peuy, K. (2008). Effects of cytokinin types and concentrations on growth and development of cell suspension culture of oil palm. Journal of Agricultural Technology 4(2): 157-163.

Compact embryogenic tissues (CET) obtained after 6-8 months of periodic subculture on callus proliferation medium (CPM), and brought to liquid CPM supplemented with 0.1 mg/l dicamba alone or in combination with 0.1, 0.5 and 1.0 mg/l BA or KN. After 6 times of subculture (2-3 weeks interval) dicamba with KN (0.1 mg/l) gave small aggregates composed of round, dense in cytoplasm led to establishment of fine suspensions in short period of time. The two PGR were proved to play an important role in both cell viability and formation of chloroplasts in embryogenic cell suspension culture.

Introduction

Cell suspension cultures have been alternatively used in oil palm propagation. Touchet *et al.* (1991) reported the culture of meristematic clumps in liquid medium for regeneration of somatic embryos. Teixeira *et al.* (1995) was success in establishment of cell suspension from long-term maintenance of friable embryogenic tissue. However, high concentration of 2,4-D (475-500 μM) or picloram (250 μM) were used at callus induction stage. This situation is very dangerous for clonal propagation of oil palm due to a huge variation. The advantage of using cell suspension culture is that culture parameters, such as pH, temperature, dissolve oxygen and agitation can be optimized and controlled. Therefore, it is possible to promote or accelerate mass propagation of oil palm in a short time to keep pace with high demand of oil palm seedling. To minimize variation of somatic embryos in cell suspension culture phytohormones both in callus and cell suspension induction should be as low as possible. Generally, 2,4-D or picloram are successfully used in both callus and cell suspension induction (Touchet *et al.*, 1991; Teixeira *et al.*, 1995). On

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

the other hands, dicamba is one of the most potent auxin that has been reported to be very effective for embryogenic callus induction and plantlet regeneration from oil palm (Te-chato *et al.,* 2004). Oil palm culture do not require exogenous cytokinins for growth, and growth is not stimulated by adding cytokinins (Nwanko and Krikorian, 1983). However, the addition of 1-5 mg/l BA increased germination rates of embryos in embryogenic suspension up to 70% (Aberlance-Bertossi *et al*., 1999). Among cytokinin KN, BA and 2-iP at very low concentration were added to proliferation medium (Rabechault and Martin, 1983).

Generally, cytokinins promote cell division and cell expansion in plant cell culture. In clonal propagation of tropical fruit trees, growth is stimulated by adding a cytokinin to a proliferation medium. Many authors have been reported suitable cytokinin types and their concentration for fruit crop species (Te-chato and Lim, 1999; 2000). Cytokinins are classified into two major groups: synthetic phenylurea derivatives, such as 1-phenyl-3-(1,2,3-thiadiazol-5-yl) urea known as thidiazuron (TDZ) and *N*-(2-chloro-4-pyridyl)-*N'*-phenylurea (CPPU), and adenine derivatives, which may occur naturally, such as kinetin (KN) and 6-benzyladenine (BA). Synthetic phenylurea derivatives, especially TDZ, have activities higher that adenine derivative (Mok *et al.*, 1987; 2000). BA is used to micropropagate a wide array of plant species, including woody plants, because of its great ability to stimulate shoot proliferation and regeneration (Montoro *et al*., 1992; Verdeil *et al*., 1994). So far, there have no comparison reports on the effectiveness of cytokinin in establishement of cell suspension culture of oil palm. This research project was to compare the response of cells in suspension culture to different cytokinin, BA and KN in terms of proliferation and development of chloroplasts.

Materials and methods

Cell suspension induction

The plant material used in this study was collected at Agricultural College, Trang and Price of Songkla University research station, Thepa. Young leaves of elite "Tenera" hybrid plant were cultured on callus induction medium by the methods described by Te-chato *et al.* (2004). Compact embryo-genic tissues (CET) were obtained after 6-8 months of periodic subculture on callus proliferation medium (CPM). This medium was MS supplemented with 3% sucrose, 200 mg/l ascorbic acid and 0.1-1 mg/l dicamba. Suspension cultures were established by placing one gram fresh weight of CET in 30 ml of liquid CPM contained in 125 ml Erlenmeyer flasks. Cultures were grown under dim

illumination of 500 lux at 26-27^{o}C on rotary shaker at 100 rpm. Rapidly growing cell suspensions were maintained by regular subculture 2-3 week intervals.

Growth determination

Four culture replicates in 125 ml Erlemeyer flasks were randomly selected for calculating fresh weight. Growth curves were based on packed cell volume (PCV) measured by the cell clumps collected in 15 ml centrifigation tubes.

Viability determination

Viability was assessed using fluorescein diacetate (FDA) as a test of membrane integrity and internal diesterase activity. After one week of culture cells in the suspension were collected by wide-bore gradual pipette. The cells were mixed with in 0.01% (w/v) FDA in the same volume and left or 10-15 minutes. The cells were then observed under ultraviolet or green light using fluorescence microscope. The percent viability was calculated as the number of cells fluorescing green per total number of cells existing x 100.

Chloroplast determination

Chloroplast formation of the cells in suspension culture was assessed using inverted microscope. The cells in suspension culture at one week after subculture were collected by wide-bore gradual pipette, transferred to petri-dish and then observed under microscope. The percent chloroplast formation was calculated as the number of cells generating chloroplasts per total number of cells existing x 100. The number of chloroplasts was counted and averaged from all cells generating one.

In the first experiment, 0.1, 0.5 and 1.0 mg/l BA or KN conentrations were tested on growth of the cells in suspension over a week period. In the second experiment, viability in those concentrations of both cytokinins were tested and chloroplast formation from the best result of each cytokinin was determined.

Results and discussion

Establishment of cell suspension culture

Establishment of cell suspensions from CET required approximately one month. Initially, the starting inoculum was passed through wide-bore pipette to eliminate large cell aggregates. The cell suspension contained both single cell

and cell aggregates composed of columnar, high vacuolar cell (Figure 1A), especially the cell in the absence of cytokinin. In the presence of KN (0.1 mg/l) small aggregates composed of round, dense in cytoplasm (Figure 1B). KN containing medium led to establishment of fine suspensions in shorter period (half) than the medium supplemented with dicamba alone. Teixeira *et al.* (1995) also described establishment of those cells in oil palm suspension cultures but the source of callus was different. They were success with FET which was obtained from log-term culture of primary globular callus (PGC) in 10 μM 2,4-D or picloram supplemented medium. However, this present study was not success in induction of cell suspension in those PGRs alone (data not shown). Addition of KN in combination with dicamba seem to convert columar or elongative cells to be round isodiametric cells and promote growth of those cells in suspension culture (Figure 2A) with doubling time as short as 7-8 days whereas BA was not effective cytokinin and gave lower result than dicamba alone (Figure 2B). Therefore, by using suitable cytokinin in liquid medium, higher rate of growth can be obtained for oil palm cell suspension culture. Aberlence-Bertossi *et al.* (1999) reported the qualitative effects of BA on establishment of cell suspension culture and embryo differentiation of oil palm. Although BA is generally used to micropropagated a wide array of plant species, including plantation crops (Montoro *et al*., 1992; Verdeil *et al*., 1994; Dhed'a *et al*., 1991), due to its great ability to stimulate shoot, it failed to enhance growth of the cell in suspension culture in this study.

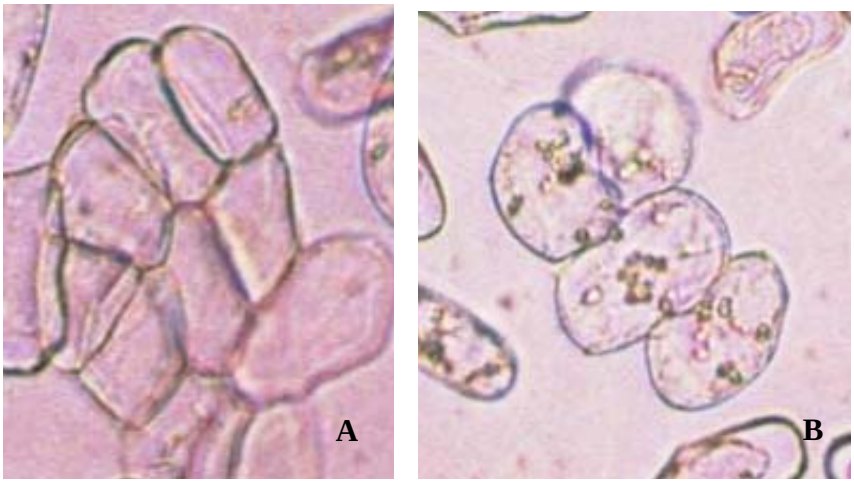

Fig. 1. The feature of cells in suspension culture of oil palm CET in MS medium supplemented with 3% sucrose, 200 mg/l ascobic acid and PGR as following;.
A: 0.1 mg/l dicamba supplemented medium
B: 0.1 mg/l dicamba and 0.1 mg/l KN supplemented medium

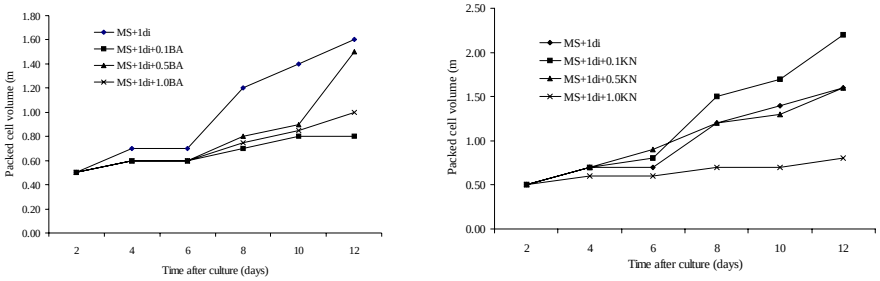

Fig. 2. Effect of BA (A) and KN (B) on growth of the cells in suspension culture in comparison with dicamba alone in MS medium supplemented with 3% sucrose, 200 mg/l ascorbic acid

Viability and differentiation of cells in suspension culture

Viability of the cells in suspension culture varied among dicamba alone and dicamba in combination with BA or KN. Dicamba alone resulted in the lowest viability of the cells in cell suspension while the combination with 0.1 mg/l KN gave the highest response. BA at higher concentration (0.5 mg/l) improved viability of the cells but lower than those obtained in 0.1 mg/l KN containing medium. Even dicamba alone gave higher PCV than dicamba in combination with BA it seems that those cells come out from periphery of original clumps by shearing force of shaking. The cells were columnar in shape and had high vacuolar as mentioned earlier with low viability and meristematic activity. Cytokinin, especially, KN altered those cells to be round shape and dense in cytoplasm which isodiametric (60-80 μm) giving rise to viable cells.
No oxidation and phenolic compound accumulation in the suspension medium. These might be due to the activity of ascorbic acid (200 mg/l) containing in the medium. CET suspension cultures maintained in dicamba containing medium displayed similar color to the medium supplemented with both dicamba and cytokinin (BA or KN). Morphology of the cells was visually distinguished by their shapes and the presence of chloroplasts. Although callus induction, proliferation and further development of somatic embryo on solidified medium have not a required exogenous cytokinins (Te-chato *et al.*, 2004) but kinetin was proved to play an important role in those activities from embryogenic suspension culture in this present study. Moreover, KN was proved to be necessary for generation of chlorophyll in those cells. The mechanisms of KN or cytokinin on chloroplast formation was not clearly understood. It might involve in cell division and some protein synthesis in relation with chloroplast development.

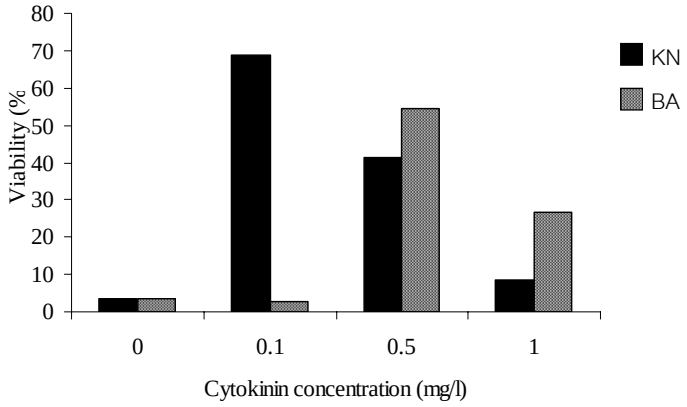

Fig. 3. Effect of BA and KN on viability of the cells in suspension culture in MS medium supplemented with 3% sucrose, 200 mg/l ascorbic acid.

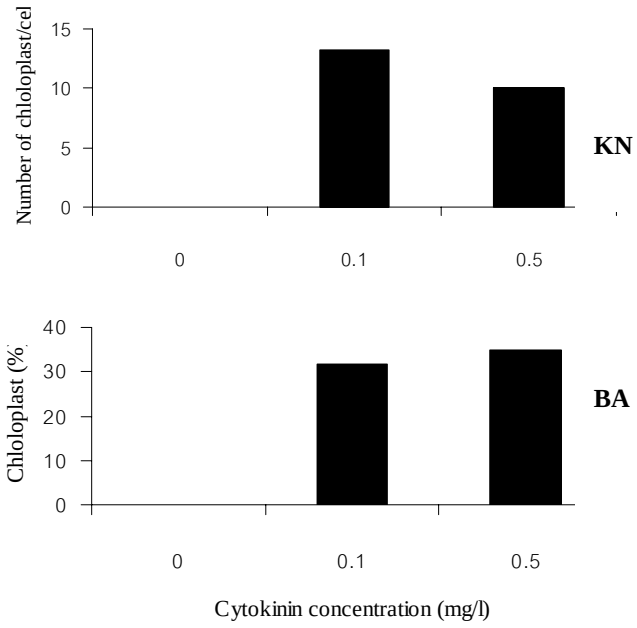

Fig. 4. Effect of different kinds and concentration of cytokinin on formation of chloroplast in cell suspension culture in MS medium supplemented with 3% sucrose, 200 mg/l ascorbic acid**.**

Acknowledgement

This project was partly supported by Prince of Songkla University (PSU) and graduate school of PSU.

References

Aberlence-Bertossi, F., Noirot, M. and Duval, Y. (1999). BA enhances the germination of oil palm somatic embryos derived from embryogenic suspension cultures. Plant Cell Tissue and Organ Culture 56:53-57.

Dhed'a, D., Dumortier, F., Panis, B.,Vuylsteke, D. and De Langhe, E. (1991). Plant regeneration in cell suspension cultures of the cooking banana cv. Bluggoe (*Musa* spp. ABB group). Fruits 46:125-135.

Mok, M.C., Mok, D.W.S., Turner, J.E. and Mujer, C.V. (1987). Biological and biochemical effects of cytokinin-active phenylurea derivatives in tissue culture systems. HortScience 22: 1194-1197.

Mok, M.C., Martin, R.C. and Mok, D.W.S. (2000). Cytokinin: biosynthesis, metabolism and perception. *In Vitro* Cellular Development of Biology Plant 36: 102-107.

Montoro, P., Etienne, H., Carron, M.P. and Nougarede, A. (1992). Incidence des cytokinines sur I induction de I embryogenese et la qualite' des embryons somatiques chez *Hevea brasiliensis* Mull. Arg. C.R. Acad. Sci. Paris 315:567-574.

Nwanko, B.A. and Krikorian, A.D. (1983). Morphogenetic potential of embryo and seedling derived callus of *Elaeis guineensis* Jacq. Var *pisifera* Becc. Annals of Botany 51: 65-76.

Rabechault, H. and J.P. Martin. (1983). Multiplication vegetative du palmier a huile (*Elaeis guineensis* Jacq.) a l' adie de culture de tissus foliaires. C.R. Academic Science 283: 1735-1737.

Te-chato, S. and Lim, M. (2000). Improvement of mangosteen micropropagation through meristematic nodular callus formation from in vitro-derived leaf explants. Scientia Horticulturae 86:291-298.

Te-chato, S. and M. Lim. (1999). Plant regeneration of mangosteen via nodular callus formation. Plant Cell Tissue and Organ Culture 59: 89-93.

Te-chato, S., H. Aslan and Y. Ibrohem, (2004). Induction of embryogenic callus and plantlet regeneration from young leaves of oil high yielding oil palm. Songklanakarin Journal Science and Technology 26: 617-628.

Teixeira, J.B., Sondahl, M.R., Nakamura, T. and Kirby, E.G. (1995). Establishment of oil palm cell suspension and plant regeneration. Plant Cell Tissue and Organ Culture 40:105-111.

Touchet, B. de, Y. Duval and C. Panettier. (1991). Plant regeneraation from embyogenic cell suspension cultures of oil palm (*Elaeis guineensis*). Plant Cell Reports 10: 529-532.

Verdeil, J.L., Huet, C., Grosdemange, F and Buffard-Morel, J. (1994). Plant regeneration from cultured immature inflorescences of coconut (*Cocos nucifera* L.): evidence for somatic embryogenesis. Plant Cell Reports 13:218-221.

(Received 14 July 2008; accepted 22 October 2008)