ORIGINAL ARTICLE

Effect of applied currents to growth in oil palm (*Elaeis guineensis* Jacq.) tissue cultures

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Abstract

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External currents of ± 2 µA were applied to the calluses of oil palm in three different culture medium recipes containing different growth substances and the effects of the current were investigated. The three media were medium for callus growth, embryogenetic medium, and organogenetic medium. The stimulation of callus growth was found for both directions of current in one unit of experiments where the embryogenetic culture medium contained NAA. In other cases the use of current seemed to have no significant stimulation effect. The mechanism by which the current may cause the alignment of the cells to promote polar transport of NAA which then increase growth and the relationship of these findings to earlier reports of the stimulation of growth in tobacco callus cultures is discussed.

Key words : applied currents, auxin, oil palm, polar transport, tissue cultures

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ผลของกระแสไฟฟ้ากับการเติบโตในการเพาะเลี้ยงเนื้อเยื่อปาล์มน้ำมัน

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ผ่านกระแสไฟฟ้า ± 2 ไมโครแอมแปร์เข้าไปในแคลลัสของปาล์มน้ำมันที่เลี้ยงในอาหารสูตรต่าง ๆ สามสูตรที่มีสารควบคุมการเจริญเติบโตต่างกันและพิจารณาผลที่เกิดขึ้น โดยอาหารสามสูตรที่ใช้นั้นเป็นสูตรสำหรับชักนำการเกิดแคลลัสการเกิดเอ็มบริออยด์และการเกิดยอดหรือราก พบว่ากระแสไฟฟ้าทั้งสองทิศทางสามารถกระตุ้นการเติบโตของแคลลัสในหนึ่งหน่วยการทดลองที่ใช้อาหารสูตรชักนำการเกิดเอ็มบริออยด์ที่มี NAA เป็นสารควบคุมการเจริญเติบโต ในกรณีอื่น ๆ กระแสไฟฟ้าดูเหมือนจะไม่มีผลในการกระตุ้นการเจริญเติบโตอย่างมีนัยสำคัญ ได้พิจารณากลไกที่กระแสไฟฟ้าอาจจะทำให้เกิดการเรียงตัวของเซลล์ซึ่งช่วยเสริมโพลาร์ทรานสปอร์ต (polar transport) ของออกซินเป็นผลให้มีการเติบโตเพิ่มขึ้น และพิจารณาความสัมพันธ์ระหว่างผลการวิจัยนี้กับงานวิจัยของผู้อื่นที่ทำมาก่อนซึ่งพบว่ากระแสไฟฟ้าสามารถกระตุ้นการเติบโตในการเพาะเลี้ยงเนื้อเยื่อยาสูบ

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Callus cultures, given suitable conditions, may lead to the differentiation of several plantlets. The main obstacle for some plants is that their calluses are rather difficult to differentiate. One of these is the oil palm (*Elaeis guineensis*).

At present, one of the questions which still requires an appropriate answer is, how do living things know where the differentiation should take place and into what form. For example, in callus cultures, how does the callus from tissue culture know whether to differentiate into shoot or into root? Several controls are thought to be involved but one of the most interesting factors may be the small steady ionic currents which have been found to be associated with the development of living things (Nuccitelli, 1990).

These small ionic currents have been measured in several developing plant and animal systems (Nuccitelli, 1990). These include the somatic embryos of carrot, *Daucus carota* (Brawley *et al*., 1984; Gorst *et al*., 1987; Rathore *et al*., 1988; Rathore and Robinson, 1989), and haploid somatic embryos of tobacco pollen (Overall and Wernicke, 1986). The current patterns in these two types of embryo were similar, that is, the current entered the cotyledon and left from the radicle. Another interesting point is that, in the embryos of the fast growing line, the currents entered both the cotyledon and the radicle and left from the middle region of the embryo (Rathore *et al*., 1988; Rathore and Robinson, 1989). These observations, together with the current patterns observed in other systems, suggested that ionic currents may be involved in the growth and development of these systems. From this idea, several studies were carried out by applying current and/or electrical fields from external sources to living systems and some effect on development was found for both plants and animals (Peng and Jaffe, 1976; Borgens, 1989; Wang *et al*., 1989). Recently this application was used in plant biotechnology with some success. Protoplast aggregation and subsequent embryogenesis were stimulated by imposed electrical fields in the mesophyll protoplasts of *Medicago sativa* (Dijak *et al*., 1986). The passing of currents of 1-2 μA from an external source through the calluses of tobacco stimulated their growth (Goldsworthy and Rathore, 1985; Rathore and Goldsworthy, 1985a; Mihai *et al*., 1994) and shoot differentiation (Rathore and Goldsworthy, 1985b). Therefore, it would be interesting to see whether the application of current from external sources

to a callus culture of oil palm, which is a slow growing plant, may help to stimulate growth and differentiation.

The purpose of this work was to investigate the effect of the low level DC currents from an external source on the growth and development of the oil palm (*Elaeis guineensis* Jacq.) callus culture. This was studied in three different media containing different growth regulators which had been found earlier to be suitable for callus growth, embryogenesis, and organogenesis (Chourykaew and Kanchanapoom, 1996; Patcharapisutsin and Kanchanapoom, 1996).

Material and Methods

Callus cultures were initiated from embryos of mature seeds of the oil palm (*Elaeis guineensis* Jacq.) on Y3 medium (Eeuwens, 1978) supplemented with 2.0 mg/l 2,4-D (2,4-dichlorophenoxyacetic acid). The cultures were subcultured every four weeks. After two months the calluses with similar colour and texture were selected and transferred to media containing various combinations of substances as follows.

Experiments on callus growth: The calluses were transferred to modified MS medium (Murashige and Skoog, 1962) designated MS-1, supplemented with 5.0 mg/l of 2,4-D and 0.05% (w/v) activated charcoal (AC). The MS-1 medium contained a half strength of the macro elements and chelating iron of MS medium, with the addition of 170 mg/l $NaH_2PO_4.H_2O$, 40 mg/l adenine sulfate, 100 mg/l casein hydrolysate, 4.5% (w/v) sucrose and 0.15% (w/v) Gelrite™. It has been found that in this medium the callus grew slowly with about 50% increase in fresh weight in two months (Chourykaew and Kanchanapoom, 1996). Therefore, the electric current was applied continuously to each of the calluses for 30 days to see whether it could stimulate similar growth in a shorter time.

Experiments on embryogenetic medium: The calluses were subcultured every four weeks for two months on modified MS medium designated MS-2, supplemented with 30 mg/l NAA (α-naphthaleneacetic acid) and 0.05% (w/v) AC. The MS-2 medium was the MS-1 without adenine sulfate and with higher amounts of thiamine-HCl, pyridoxine-HCl, 0.4 and 0.5 mg/l respectively. For embryoid production, the calluses were transferred to the MS-2 medium with 70 mg/l of NAA and 0.05% (w/v) AC. The current was then applied continuously to each of the calluses for two months, the same period for embryoid induction without current (Patcharapisutsin and Kanchanapoom, 1996), to see whether the applied current could stimulate the formation of more embryoids.

Experiments on organogenetic medium: The calluses were subcultured every four weeks for two months on MS-1 medium supplemented with 5.0 mg/l 2,4-D and 0.05% (w/v) AC. For shoot and/or root initiation the calluses were transferred to MS-1 medium supplemented with 15% (v/v) coconut water and 0.05% (w/v) AC. It was found that in this medium the differentiation of shoot like growth structure would also take about two months (Patcharapisutsin and Kanchanapoom, 1996). The current was then applied continuously for two months to see whether it could stimulate the forming of more root and/or shoot.

Current application: The callus was placed on 25 ml of the experimental medium in a 125 ml conical flask covered with a rubber plug. A small glass tube (1 mm diameter, 80 mm length) was pushed through a small hole in the center of a rubber plug and the larger hole (5 mm diameter) on the side was filled with cotton wool. The two electrodes were made from 300 mm lengths of Teflon® coated stainless steel wire with an uninsulated diameter of 0.25 mm obtained from Cooner Wire Company, Chatsworth, USA. Five mm of Teflon® from both ends was peeled off. One electrode was placed in the medium towards the side of the flask and was bent over the rim of the flask. The other wire electrode was threaded through the glass tube in the center of the rubber plug and emerged a few mm above the medium. The flasks containing the medium and the rubber plugs together with the electrodes were autoclaved and left to cool. Calluses weight between 400-700 mg were transferred under sterile conditions, the wire electrode in the center of the rubber plug was in-

serted about 2 mm into the callus which was then placed on the medium inside the flask. The rubber plug was then pressed firmly onto the top of the flask and covered with aluminium foil. In each experiment the flasks were divided into three groups (Figure 1) as follows:

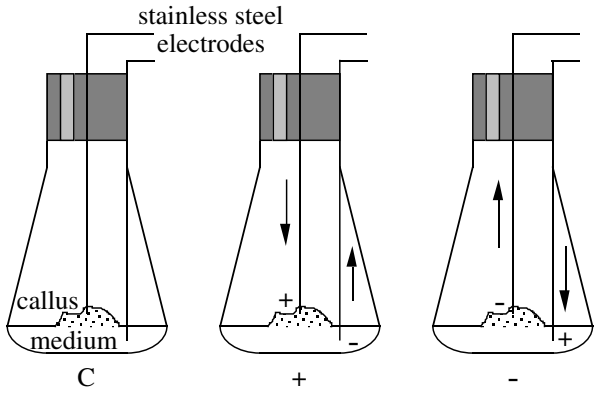

Figure 1. Experimental set up for the stimulation of oil palm callus. C: control group, no current was passed; +: positive group, electrode inside the callus was connected to the positive potential of the power supply and the current passed through the callus into the medium; -: negative group, the reverse of +.

C group	control, electrodes were not connected to the power supply
+ group	the wire electrode in the callus was connected to the positive potential of the power supply (VCC 600, Voltage-Current Clamp, Physiologic Instruments, San Diego, USA)
- group	the reverse of + above

Each group had 10-15 flasks, *i.e*. 10-15 replications. The experiment was repeated at least three times for each of the three media. All experiments were done at 26 ± 1 °C with 16 h photo period of Gro-Lux light at 2000 lux .

The current from the power supply had to pass through a 4.7 MΩ resistor in series with the callus. The change in resistance of the callus (if any) during the experiment was very small compared to this resistance and, hence, there would be very little effect on the amount of current set for the experiment. The current was checked intermittently throughout the experiment and the change was generally less than 2%.

Since the average ionic current density around a germinating oil palm embryo during the early developmental stages was found to be between 1-2 μA/cm^2 (Thavarungkul, 1997) and the calluses used in the experiments were prepared to have a contact area with the medium of about 1 cm^2, the current of 2 μA was chosen to pass through each callus. The current density in the callus decreased from about 120 μA/cm^2 adjacent to the electrode to about 2 μA/cm^2 at its interface with the medium and this would decrease during the 1-2 months experimental period as the callus grew in size. In all experiments the parameter evaluated was the fresh callus weight. For each experiment the averages of the percentage weight increase and the standard deviations (SD) of each of the three groups (C, + and - groups) were calculated and the results were analysed using one-way ANOVA (analysis of variance). (For details of the method of analysis see e.g. Sokal and Rohlf (1981)

For the experiments on embryogenetic and organogenetic media, the number of calluses with embryoid, shoot and/or root were also counted. A statistical test for the factors which might have some effect on embryogenesis or organogenesis was done using the G-test (Sokal and Rohlf, 1981).

Results

Experiments on callus growth: Figure 2 shows the effects of passing 2 μA currents on callus growth. The analysis, using ANOVA at the 0.05 level, showed that the differences in the percentage increase in callus weight between calluses with and without current are not significant ($p>0.05$). It can be seen from the results that the SD of each group in all experiments is rather high. This is probably due to the nature of the oil palm which normally has a high variation between its embryos which then causes the high variation between the calluses even when the calluses were

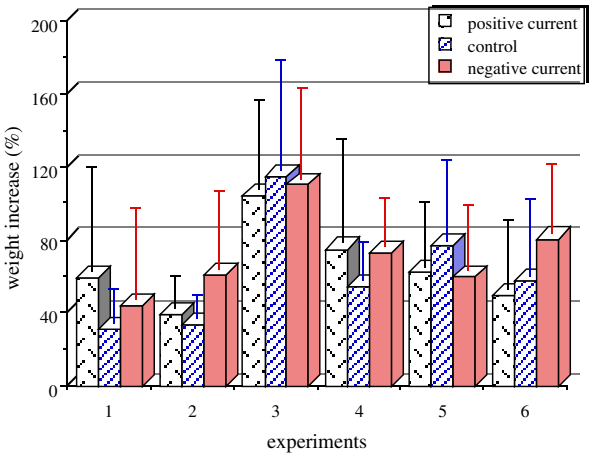

Figure 2. Effect of passing 2 µA of current for 30 days on the growth of oil palm callus on modified MS medium containing 5.0 mg/l of 2,4-D and 0.05% (w/v) activated charcoal. Bars show standard deviation of the data.

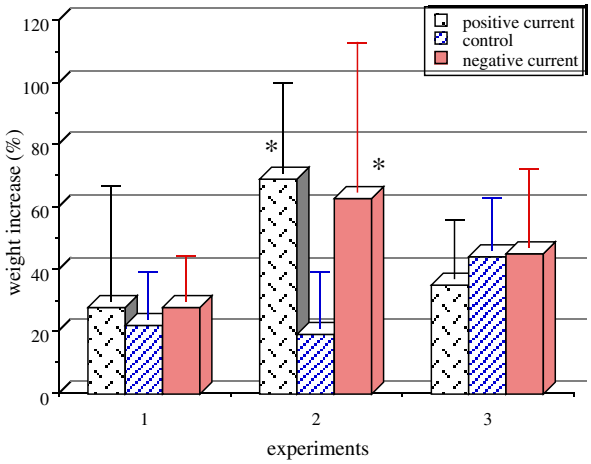

Figure 3. Effect of passing 2 µA of current for 2 months on the growth of oil palm callus on modified MS medium containing 70 mg/l of NAA and 0.05% (w/v) activated charcoal. * indicates results which are significantly different from controls at p<0.05. Bars show standard deviation of the data.

induced from embryos with very similar features. Therefore, different experiments, where calluses were induced from different sets of embryos, would result in more variability. The variations between experiments was confirmed by the analysis using two way ANOVA between experiments 1-6 and it was found that they varied significantly ($F = 10.73$, $p < 0.05$).

Experiments on embryogenetic medium: After passing 2 µA currents through the calluses inoculated in the medium for embryoid initiation containing 70 mg/l NAA for two months the percentages of callus growth were as shown in Figure 3. The ANOVA test on each of the three experiments showed that only in the second experiment were the increases in weight of the positive and negative current groups significantly different from the control ($F = 4.66$, $p < 0.05$). The test also showed significant variation between the experiments ($F = 3.83$, $p < 0.05$). The test on the number of calluses with embryoids failed to show that the currents had any effect on the initiation of embryoids (G-value = 3.38, dF =2, $p > 0.05$).

Experiments on organogenetic medium: Initially oil palm calluses were cultured in a medium with high auxin because a reduction in auxin resulted in embryogenesis or organogenesis (Krikorian *et al*., 1987). Earlier experiments (Patcharapisutsin and Kanchanapoom, 1996) showed that calluses of oil palm in a medium devoid of NAA and supplemented with 15% (v/v) coconut water (CW) would produce young plantlets while the absence of CW would halt growth. This may be due to the fact that CW is the liquid endosperm which contains the various nutrients necessary for growth e.g. indole acetic acid, cytokinin, giberellins *etc*. (Naylor, 1984). In addition since coconut and oil palm are in the same family CW is, therefore, suitable for embryogenesis and organogenesis of the oil palm.

In this experiment the currents were applied for two months and the results are shown in Figure 4. The ANOVA test at 0.05 level for each experiment indicated that the different nature of the currents had no significant effect on the weight increase but there are significant variations between experiments ($F = 9.01$, $p < 0.05$).

From the experiment it was found that some

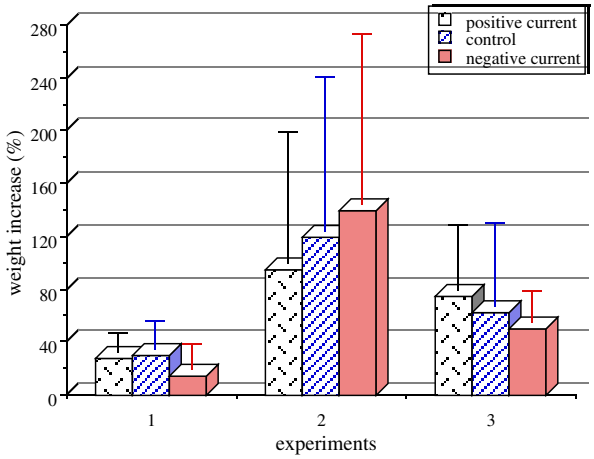

Figure 4. Effect of passing 2 µA of current for 2 months on the growth of oil palm callus on modified MS medium containing 15% (v/v) coconut water and 0.05% (w/v) activated charcoal. Bars show standard deviation of the data.

calluses only produced roots, some produced shoots and a few produced both shoots and roots. The analysis using the G-test indicated that there is no significant relationship between the use of current and the initiation of shoot and/or root (G-value = 2.42, dF = 2, $p > 0.05$).

Discussion

The stimulation of living systems using external current sources has been found to produce various effects such as movement and growth, or to set up polarity for growth (Nuccitelli, 1988). It was found that current from an external source stimulated callus growth in tobacco by 70 percent (Goldsworthy and Rathore, 1985; Rathore and Goldsworthy, 1985a) and also stimulated the forming of a shoot in the callus 5-fold (Rathore and Goldsworthy, 1985b). The interest in this study is the stimulation of growth in the normally slow-growing calluses of oil palm (*Elaeis guineensis* Jacq.). However, there was only one experiment (Figure 3) in this work which indicated that currents stimulated growth in callus on medium containing NAA. Part of the reason may due to the high variation between the oil palm calluses, though they were selected to have similar colour and texture. This can be observed by the large values of the SD's.

The absence of a significant stimulation effect of currents on the callus cultures in almost all of the cases investigated raises the question of whether: (1) the currents applied were insufficient, (2) the growth regulators (auxin) were not suitable, (3) electric field or electric current might play a less important role in oil palm callus cultures compared to tobacco cultures, or (4) the electrode products created an inhibitory effect.

The first possibility seemed unlikely since the natural ionic currents found in germinating oil palm embryos were of the same order as that used in this work (Thavarungkul, 1997). If higher current density was required our experimental set up had created a gradient of the current density from the area next to the electrode in the callus (120 µA/cm^2) to the callus-medium interface (2 µA/cm^2) (this range was used in tobacco callus stimulation (Goldsworthy and Rathore, 1985; Rathore and Goldsworthy, 1985a; Rathore and Goldsworthy, 1985b)), therefore, there should be some region within the callus where the current density would be most suitable. This argument was supported by the work of Rathore and Goldsworthy (Rathore and Goldsworthy, 1985b) where shoots were formed in the most negative region of the culture. The period of 1-2 months in which the currents were applied would also be sufficient to be able to see the effect (if any) since this was the normal period in which growth and development of calluses in these media were observed in the absence of applied current (Chourykaew and Kanchanapoom, 1996; Patcharapisutsin and Kanchanapoom, 1996). For the experiments on organogenesis, the calluses were maintained for a few more months after the end of the experiments; however, no further stimulation effect was observed.

Goldsworthy and Rathore (1985) found that the synthetic auxin 2,4-D would not stimulate growth in tobacco cullus and only the natural auxin IAA (indole acetic acid) facilitates the stimulation of growth. However, Mihai *et al*. (1994) found that

stimulation effect was obtained in the presence of another synthetic auxin NAA. In this work we have tried both the synthetic auxin (2,4-D and NAA) and natural auxin (IAA within the coconut water (Naylor, 1984)) and it is interesting that no stimulation effect was observed. Why did the different type of auxin show different effects on the stimulation of callus cultures?

The exact mechanism of external applied current in stimulating growth and/or changes in biological systems is still uncertain. It is possible that the current which flows between the medium and the callus helps to transport auxin anions into the callus. If this is the case, the extra growth should only be observed when the positive electrode is inside the callus (positive current). In most of the experiments, however, both positive and negative currents provided no significant growth increase over control where no current was passed. Moreover, if this hypothesis is correct the use of 2,4-D should provide more growth than NAA or IAA because 2,4-D is more acidic (pK values of 2,4-D, NAA and IAA are 2.8, 4.2 and 4.7 respectively (Rubery, 1987)) and, thus, there should be more negative ions of 2,4-D than of NAA and IAA. However, currents had no effect on growth when 2,4-D was used, both in this work (Figure 2) and the work of Goldsworthy and Rathore (1985). On the contrary, IAA and NAA has more ability to induce growth when stimulated with current (Goldsworthy and Rathore, 1985; Rathore and Goldsworthy, 1985a; Rathore and Goldsworthy, 1985b; Mihai *et al*., 1994). Therefore, this hypothesis seems very unlikely.

The preferred hypothesis is the one proposed by Goldsworthy and Rathore (1985) i.e. the applied current would help to align the physiological polarities of the callus cells so as to promote the polar transport of auxin. In polar transport, auxin moves through a section of plant tissue more rapidly in one direction than the other. This movement depends on the pH gradients, membrane potentials, and the difference in permeability ratio for the apical and basal membranes (Goldsmith, 1977; Lomax *et al*., 1995). The external applied current and associated potential gradient may act on the membrane. Since many molecules in the membrane are charged and free to move, this potential gradient would be able to redistribute or aggregate them within the plane of the plasma membrane (Jaffe and Nuccitelli, 1977). The other possibility is that the imposed field depolarizes the cathode-facing plasma membrane and hyperpolarizes the anode-facing membrane (Nuccitelli, 1988). In both cases, a potential gradient would be established within the cell resulting in the accumulation of differently charged molecules, possibly including auxin anions, at opposite sides of the cell. Goldsworthy and Rathore (1985) suggested that a change in the direction of the cell's electrical polarity would be expected to result in a corresponding change in its direction of growth as well as the direction of auxin transport. This is consistent with their results where only negative current caused significant growth while positive current either caused no significant growth or slightly inhibited growth.

The chemiosmotic hypothesis for polar auxin transport proposed that the undissociated auxin moves across the cell membrane into the cytoplasm where it dissociates into auxin anion and H^+ (Goldsmith, 1977; Lomax *et al*., 1995). This suggests that before the current can help to stimulate growth by setting up the polar transport of auxin anion, the auxin from the medium must permeate into the cells of the callus. It has been found that the uptake of the stronger acidic auxins is less than that of the weaker acidic auxins (Goldsmith, 1977), hence, between 2,4-D, NAA and IAA the uptake of 2,4-D would be the least and that of IAA the most. This might be the reason why only NAA and IAA were found to stimulate growth in tobacco cultures (Goldsworthy and Rathore, 1985; Rathore and Goldsworthy, 1985a; Rathore and Goldsworthy, 1985b; Mihai *et al*., 1994). In addition, the uptake of auxins also depends on the permeability of the specific tissue to auxin (Rubery, 1987). Therefore, for oil palm callus, which is normally very slow in growing (Corey *et al*., 1977), it is possible that the permeability of the callus to auxin is very low compared to tobacco and the application of external current

would have little effect due to the little amount of auxin inside the callus.

Another possibility why external current has very little effect on oil palm in contrast to tobacco tissue cultures is that oil palm is a tree while tobacco is herbaceous and these two plants have different abilities to grow. Therefore, the stimulated effect of current on tobacco may not be applicable to oil palm due to the different nature of these plants. However, further studies are necessary, for example to find whether the absence of the stimulation of growth by external applied currents would also be observed in tissue cultures of other trees such as coconut palm or rubber tree.

It is also interesting to see that tobacco callus treated with alternating electric current also showed stimulation of growth on medium containing NAA (Mihai *et al*., 1994). In this case, the above hypothesis is not operative since the polarity of the callus cells is periodically changed and therefore, another mechanism must be involved. Mihai *et al*. (1994) suggested that the effects are due to a high sensitivity of the callus cells resulting in the electrical stimulation of some membrane processes which then stimulated growth. From this it is most likely that the stimulation of growth by applied current or applied electric field may involve more than one mechanism.

Finally, there were some arguments that the use of stainless steel electrodes leads to an "electrode inhibitory effect" (Mihai *et al*., 1994). However, there were other works where stainless steel electrodes were used to apply currents to the callus cultures and it could be concluded with reasonable certainty that the stimulation of growth and shoot regeneration was due to the electrical fields set up by the passage of the current (Goldsworthy and Rathore, 1985; Rathore and Goldsworthy, 1985b). In this work some electrode products might exist in the callus since the currents was applied over a long time. However, the calluses used were quite large (400-700 mg) and there must be some area of the callus where the current was passing through without the effect from the electrode products. Therefore, this factor seemed rather unlikely to be a significant contributor to the negative results.

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References

Borgens, R.B. 1989. Natural and applied currents in limb regeneration and development. **In** R. B. Borgens, K. R. Robinson, J. W. Vanable Jr and M. E. McGinnis (Eds.) Electric Fields in Vertebrate Repair: Natural and Applied Voltages in Vertebrate Regeneration and Healing. Alan R. Liss, Inc., New York. 27-75.

Brawley, S.H., Wetherell, D.F. and Robinson, K.R. 1984. Electrical polarity in embryos of wild carrot precedes cotyledon differentiation. Proc. Natl. Acad. Sci. USA, 81: 6064-6067.

Chourykaew, B. and Kanchanapoom, K. 1996. *In vitro* culture of embryos and callus of oil palm (*Elaeis guineensis* Jacq.). J. Sci. Soc. Thailand, 22: 1-12.

Corey, R.H.V., Barret, J.N. and Jones, L.H. 1977. Vegetative propagation of oil palm via tissue culture. Oil Palm News, 22: 2-7.

Dijak, M., Smith, D.L., Wilson, T.J. and Brown, D.C.W. 1986. Stimulation of direct embryogenesis from mesophyll protoplasts of Medicago sativa. Plant Cell Rep., 5: 468-470.

Eeuwens, G.J. 1978. Effects of organic nutrients and hormones on growth and development of tissue explants from coconut (*Coco nucifera*) and date palms (*Phoenix dactylifera*) culture *in vitro*. Physiol. Plant., 42: 173-178.

Goldsmith, M.H.M. 1977. The polar transport of auxin. Annu. Rev. Plant Physiol., 28: 439-478.

Goldsworthy, A. and Rathore, K.S. 1985. The electrical control of growth in plant tissue cultures: The polar transport of auxin. J. Exp. Bot., 36: 1134-1141.

Gorst, J., Overall, R.L. and Wernicke, W. 1987. Ionic currents traversing cell clusters from carrot suspension culture reveal perpetuation of morphogenetic potential as distinct from induction of embryogenesis. Cell Differ., 21: 101-109.

Jaffe, L.F. and Nuccitelli, R. 1977. Electrical controls of development. Annu. Rev. Biophys. Bioeng., 6: 445-476.

Krikorian, A.D., Kelly, K. and Smith, D.L. 1987. Hormones in tissue culture and micro propagation. **In** P. J. Davies (Eds.) Plant Hormones and Their Role in Growth and Development. Martinus Nijhoff Publishers, Dordrecht. 593-613.

Lomax, T.R., Muday, G.K. and Rubery, P.H. 1995. Auxin transport. **In** P. J. Davies (Eds.) Plant Hormones. Kluwer Academic Publishers, Dordrecht. 509-530.

Mihai, R., Cogalniceanu, G. and Brezeanu, A. 1994. Control of *Nicotiana tabacum* callus growth by alternating and pulsed electric field. Electro. Magnetobiol., 13: 195-201.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15: 473-497.

Naylor, A.W. 1984. Functions of hormones at the organ level of organization. **In** T. K. Scott (Eds.) Hormonal Regulation of Development II. Springer-Verlag, Berlin. 172-218.

Nuccitelli, R. 1988. Physiological electric fields can influence cell motility, growth and polarity. Adv. Cell Biol., 2: 213-233.

Nuccitelli, R. 1990. Vibrating probe technique for studies of ion transport. **In** J. K. Foskett and S. Grinstein (Eds.) Noninvasive Techniques in Cell Biology: Modern Cell Biology. Vol 9. Wiley-liss, New York. 273-310.

Overall, R.L. and Wernicke, W. 1986. Steady ionic currents around haploid embryos formed from tobacco pollen in culture. Prog. Clin. Biol. Res., 210: 139-145.

Patcharapisutsin, W. and Kanchanapoom, K. 1996. Somatic embryogenesis and plantlet regeneration from oil palm (*Elaeis guineensis* Jacq.) callus. J. Sci. Soc. Thailand, 22: 13-20.

Peng, H.B. and Jaffe, L.F. 1976. Polarization of fucoid eggs by steady electric fields. Dev. Biol., 53: 277-284.

Rathore, K.S. and Goldsworthy, A. 1985a. Electrical control of growth in tissue cultures. Bio/Technology, 3: 253-254.

Rathore, K.S. and Goldsworthy, A. 1985b. Electrical control of shoot regeneration in plant tissue cultures. Bio/Technology, 3: 1107-1109.

Rathore, K.S., Hodges, T.K. and Robinson, K.R. 1988. Ionic basis of currents in somatic embryos of *Daucus carota*. Planta, 175: 280-289.

Rathore, K.S. and Robinson, K.R. 1989. Ionic currents around developing embryos of higher plants in culture. Biol. Bull., 176: 46-48.

Rubery, P.H. 1987. Auxin transport. **In** P. J. Davies (Eds.) Plant Hormones and Their Role in Growth and Development. Martinus Nijhoff Publishers, Dordrecht. 341-362.

Sokal, R.R. and Rohlf, F.S. 1981. Biometry. 2nd ed. W.H. Freeman and Company, New York.

Thavarungkul, P. 1997. Vibrating probe measurement of ionic currents around developing embryo of oil palm (*Elaeis guineensis* Jacq.). J. Exp. Bot., 48: 1647-1653.

Wang, P., Rathore, K.S. and Robinson, K. 1989. The responses of pollen to applied electric fields. Dev. Biol., 136: 405-410.