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The growth and biochemical responses on *in vitro* cultures of *Oncidium taka* orchid to electromagnetic field

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Abstract

The effects of electromagnetic fields (EMF) strengths of 0, 10, 20, 40, 60, 80, 100 and 120 kV/m were investigated using protocorm-like bodies (PLBs) of *Oncidium taka* orchid cultures under *in vitro* condition. Various biochemical and antioxidant system changes in PLBs were investigated. The results obtained reveal the potential of using 40 kV/m electric field strength stimulated production of higher photosynthetic pigments and increasing the growth of *Oncidium taka* PLBs. The results also showed the difficulties in obtaining and establishing a clear relationship between the influencing electric field and the protein, nitrogen content, peroxidase (POX) and glutamate oxaloacetate transaminase (GOT) activities. This suggests that electromagnetic field could be used as a tool to promote *Oncidium* taka orchid growth via photosynthesis once the right EMF strength and duration of exposure has been established through future studies.

Keywords: Orchid, Protocorm-like bodies, Electromagnetic field , Biochemical markers.

Abbreviations: EMF_Electromagnetic fields; **GOT**_Glutamate oxaloacetate trancaminase; **PLBs**_Protocorm-like bodies **POX**_Peroxidase; **SDS-PAGE**_Sodium dodecyl sulfate polyacrylamide gel

Introduction

Oncidium taka belonging to the family Orchidaceae is one of the many orchid hybrids available in Malaysia. Generally, the *Oncidium* sp., commonly known as "Dancing Ladies" are bright yellow flowers with red brown markings on the petals borne in shower of hundred on each spray. Through *in vitro* system, *Oncidium* sp. protocorm-like bodies (PLBs) were produced when orchid shoot apices are cultured on a semi-solid medium. The explant would turn green and slowly enlarge to form protocorms, also known as the developmental stage of the orchid embryo from the *in vitro* system. Subsequently, PLBS could be multiply into clumps when maintained in liquid media and each will give rise to a new plantlet via shoot formation upon transferring onto semi-solid media. Various research work have reported on the promising effects of both electric and magnetic field on *in vitro* plant cultures and such as increased growth and shoot formation (Matsuo et al., 1992; Matsuo and Uchino, 1993b; Magone, 1996; Kondic et al., 1998). However, insignificant effects and growth disruption have also been reported leading to inconclusive findings on the effects of electromagnetic field (EMF) on *in vitro* plants. The ability of electromagnetic field to increase shoots formation and growth and regeneration of plantlets from plant tissue cultures. Studies have shown that certain level of irradiation on the *in vitro* cultures of orchid favours growth by increasing the fresh weight of the protocorm-like bodies (PLBs) while other level had no effect or was detrimental to the PLBs (Adrain 1999). On the study of a new environmental factor such as electromagnetic field, *in vitro* plant cultures are favoured due to their ability to grow under aseptic and controlled conditions of temperature, light and nutrition. Furthermore, *in vitro* plant tissue cultures are genetically uniform and this provides a homogenous basis for physiological and biochemical experimentations. *In vitro* protocorm-like bodies (PLBs) cultures of orchid hybrid *Oncidium taka* were used in this study due to their established micropropagation method and reasonably high multiplication rates. Currently in Malaysia, no such studies have been conducted on the possible effects of electric field as a non-ionizing radiation especially on the *in vitro* plants. Tret'yakov et al. (1994) showed the ability of 5 and 10 kV/m electric field to increase the plant regenerants quality and reproduction coefficient of *in vitro* black currant by treating their apices and microsprouts. Kondic et al. (1998) reported that 8 and 15 Hz EMF for 30 minutes stimulates callus formation and growth of wheat (*Triticum aestivum* L.) while 30, 50 and 72 Hz failed to have any significant influence. In another case, *in vitro* shoots of potato (*Solanum tuberosum*) plantlets treated with different frequencies of EMF for 20 minutes resulted in a decrease in shoot length for 8 Hz and 8.7 kHz 3 weeks after culture while 2.4 kHz resulted in higher shoot length (Fartais et al., 1995). *In vitro* cultures of plant whose cells are usually grown in a homeostatic

environment are much more sensitive to changes than whole plants or unicell cultures (Mina and Oldsworthy 1992). The use of *in vitro* plant cultures such as PLBs enables the selection of uniform for morphological, physiological and biochemical studies. Therefore, this study aims to investigate the effects of different electric field strength on the growth and biochemical changes in the *in vitro* PLBS of orchid hybrid, *Oncidium taka.*

Results and Discussion

Growth studies

Table 1 showed the effects of the electric field treatment on the mean fresh weight gain of *Oncidium taka* PLBs determined at three weeks intervals for twelve weeks after treatment.

The results showed that 40 kV/m electric field treatments resulted in a significantly ($p < 0.05$) higher fresh weight gain throughout the twelve weeks of growth. At the third week after treatment, it had a fresh weight increase of approximately 1.4 times higher compared to the control and at the sixth, ninth and twelfth week, it had a fresh weight increase of 96%, 60% and 54%, respectively. The fresh weight gain of 40 kV/m treated *Oncidium taka* PLBs seems to be decreasing with time, thus, signifying the decreasing effects of the electric field on promoting PLBs growth. Besides 40 kV/m treatment, 20 kV/m electric field treatment also resulted in significantly ($p < 0.05$) higher fresh weight gain at the sixth and twelfth weeks after treatment with an increase of 52% and 27% respectively compared to the control. According to Goldsworthy and Rathore (1985), enhanced shoot formation in vitro cultures was due to the increased parallel orientation of the growth axes of individual cells stimulated by the electric field. Electric field treatment of 10 kV/m seemed to discourage the PLBs growth as the fresh weight gain of 10 kV/m were significantly ($p < 0.05$) less than the control with a reduction of 75.6%, 62.9%, 40% and 24.5% at three, six, nine and twelve weeks respectively. The percent of fresh weight reduction in treatment 10 kV/m when compared to the control were also decreasing with time, thus suggesting the reducing effect of the electric field. Therefore, Table 1 showed that treatments 10, 20 and 40 kV/m gave a more significant result compared to treatments 60, 80, 100 and 120 kV/m which were insignificantly ($p > 0.05$) different from the control (Table 1). The different PLB response to electric field of different strength is supported by Kondic *et al*. (1998) who reported that 8 and 15 Hz EMF for 30 minutes stimulates callus formation and growth of wheat (*Triticum aestivum* L.) while 30, 50 and 72 Hz failed to have any significant influence. In another case, *in vitro* shoots of potato (*Solanum tuberosum*) plantlets treated with different frequencies of EMF for 20 minutes resulted in a decrease in shoot length for 8 Hz and 8.7 kHz 3 weeks after culture while 2.4 kHz resulted in higher shoot length (Fartais et al., 1995).

Fresh weight and dry weight

The fresh weight gain in plant tissue cultures such as callus or protocorm-like bodies (PLBs) may be attributed to the increase in the moisture content or accumulation of biomass (Matsuo et al., 1992). Table 2 showed the fresh weight, dry weight and moisture content of the *Oncidium taka* PLBs twelve weeks after treatment with electric field.

Significantly ($p < 0.05$) higher mean fresh weight of 6.275 g and mean moisture content of 97.2% in the PLBs treated with 40kV/m suggests that the 40kV/m electric field treatment promotes the growth of PLBs by increasing its biomass and moisture accumulation. This was as observed by Matsuo and Uchino (1993b) who found that plant response to an optimum electric field strength for the enhancement of growth when they found only 75 kV/m electric field promoted growth in mint plantlets while lower or higher electric field inhibited growth. The enhancement or inhibition of growth was also found to be more prominent in the roots. Matsuo and Uchino (1993a) found higher callus multiplication on asparagus (*Asparagus officinalis*) with electric field intensities between 100 to 250 kV/m compared to control and the effect is independent of the exposure period. This signifies the indifference in the electric field effects within the range. The significantly ($p < 0.05$) higher moisture content of 97.2% and an insignificantly ($p > 0.05$) different dry weight in treatment 40 kV/m (Table 2) suggest that the effects of electric field on the water suction force of the callus. Matsuo et al. (1992) found that the increased growth ration of apple mint fresh weight and root length in 0.5 kV/m alternating field was due to increased water absorption. According to Bachman and Reichmanis (1973a) who experimented on barley plants, high electric fields of 200 kV/m and above retards growth while electric field below 200 kV/m enhances growth with greater enhancement at an optimum electric field of 50 kV/m. Their study showed the existence of optimum electric field strength for growth enhancement. Goldsworthy (1998) reported similar findings when direct application of electric current on tobacco callus resulted in 70% increase in growth rate. Artificially applied electric field was found to induce the polarization of cell clusters or repolarize the cells, thus increasing the level of cell coordination to build structures such as shoots. A number of researchers such as Karcz and Burdach (1995) and Desrosiers and Bandurski (1998) discovered that electric field as low as 15 V alters the transport system and distribution of auxin, thus affecting growth-inducing hormones such as indole acetic acid (IAA). According to Hager et al. (1991), electric field activities IAA and turn the activations of the protons pumping across the membrane. The hyperpolarized membrane potential then increases cell wall and volume via uptake of water and leads to cell elongation and growth. Another reason for the increased in the growth rate may be due to the high water content in the PLBs as reported by Prendeville and Battles (2001). The mechanism, which increased the growth rate of the *Oncidium taka* PLBs may be due to the energization of the water content with the electromagnetic radiation, namely high electric field. Prendeville and Battles (2001) reviewed that Dwain Morse, a Californian Scientist developed the original concept of using electromagnetic energy on water to speed up the growth rate of plants in 1995. Electromagnetic energy when passed through water, charges it like a battery and the water's energy then accelerates the photosynthesis process by mimicking the energy from the sunlight.

Table 1. Effects of electric field on the mean fresh weight gain of *Oncidium taka* PLBs

Treatments (kV/M)	Mean Fresh Weight Gain (g)			
	3 Weeks	6 Weeks	9 Weeks	12 Weeks
0	0.45bc	1.08cd	2.31b	3.55c
10	0.11d	0.40e	1.39c	2.68d
20	0.55b	1.63b	2.38b	4.53b
40	1.06a	2.11a	3.70a	5.48a
60	0.28cd	1.46bc	2.37b	3.28c
80	0.40bc	1.08cd	2.31b	3.52c
100	0.35bc	0.68de	1.87bc	3.73c
120	0.32c	0.96d	1.37c	4.29b

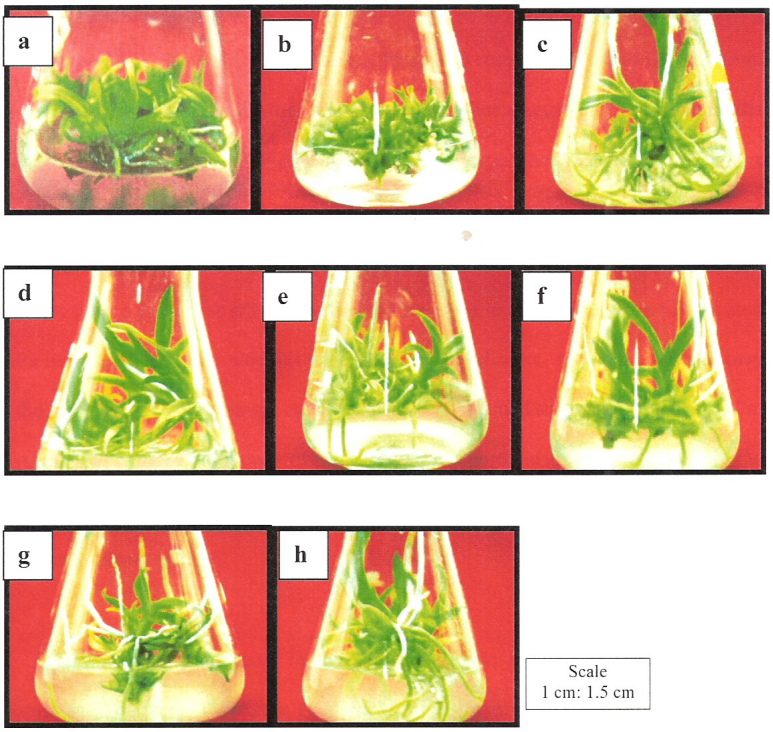


Fig 1. Effects of Electric Field Treatment on the morphology of *Oncidium taka* PLBs six months after treatment

Another novel concept by Prendeville and Battles(2001) suggests that small electromagnetic energy when applied into water releases hydrogen peroxide, which leads to the increased oxygen content in the water. This technology had been used successfully to increase the growth rate of carrot by 45% and lettuce by 33%. The technology has also been used in Britain to speed up grass growth on football pitches and claimed to increase the biomass of everything from the grass to oak tree. The electromagnetized water was also found to prolog the shelf life of cut flowers by a week and increases the vitamin C content of fruit by 10% (Prendeville and Battles 2001). Therefore, the effect of the electromagnetic field on the high water content in PLBs may have resulted in the increased growth. Table 2 showed that the 40 kV/m treated PLBs had relatively higher moisture content and less dry matter compared to the untreated PLBs. In comparison, the fresh weight of the 40 kV/m treated PLBs was found to contribute only 2.8% to the dry matter while the untreated PLBs had lower moisture content and contributed 3.9% to the dry weight. A high dry weight or low water content suggests increased solutes concentration in the cells. Among the organic osmolytes that accumulate in plants grown in stressed environment such as salinity are soluble nitrogenous compounds, organic acids, sugars and polyols (Fathi-Ettai and Prat 1990). Besides treatment 40 kV/m, PLBs treated with 20 kV/m and 120 kV/m also had significantly ($p < 0.05$) higher fresh weight than the control. However, their respective dry weight were insignificantly ($p > 0.05$) different from the control. The lowest moisture content was found in PLBs treated with 10 kV/m and also the lowest fresh and dry weight. The high growth rate of 40 kV/m treated PLBs could be visibly observed in Figure 4, which showed the PLBs after 6 weeks of growth. The results obtained in this study was as reported by Jain *et al*. (1990) who found that a high fresh weight to dry weight ratio indicates increased mitotic activity, water content and cell size. According to Table 2, the highest fresh to dry weight ratio was found in treatment 40 kV/m with 35.9, followed closely by treatment 20 kV/m with 32.1, and 100 kV/m with 30.4. Therefore, all the three treatments stated yielded high fresh weight and vigorous growth due to the increase in cell multiplication and size. Although no growth deformities were observed in the twelve weeks after treatments, some

Table 2. Effects of electric field on the mean fresh weight, dry weight, and moisture content of *Oncidium taka* PLBs 12 weeks after treatment.

Treatments (kV/M)	Mean Fresh Weight Gain (g)	Mean Dry weight x 10^{-1} (g)	Mean Moisture Content (%)
0	3.852de	1.517abc	96.5cde
10	3.482e	1.373c	96.1f
20	5.327b	1.660ab	96.9b
40	6.275a	1.746a	97.2a
60	4.077de	1.516abc	96.3ef
80	4.321d	1.541abc	96.4de
100	4.527cd	1.490bc	96.1bc
120	5.093bc	1.726ab	96.6cd

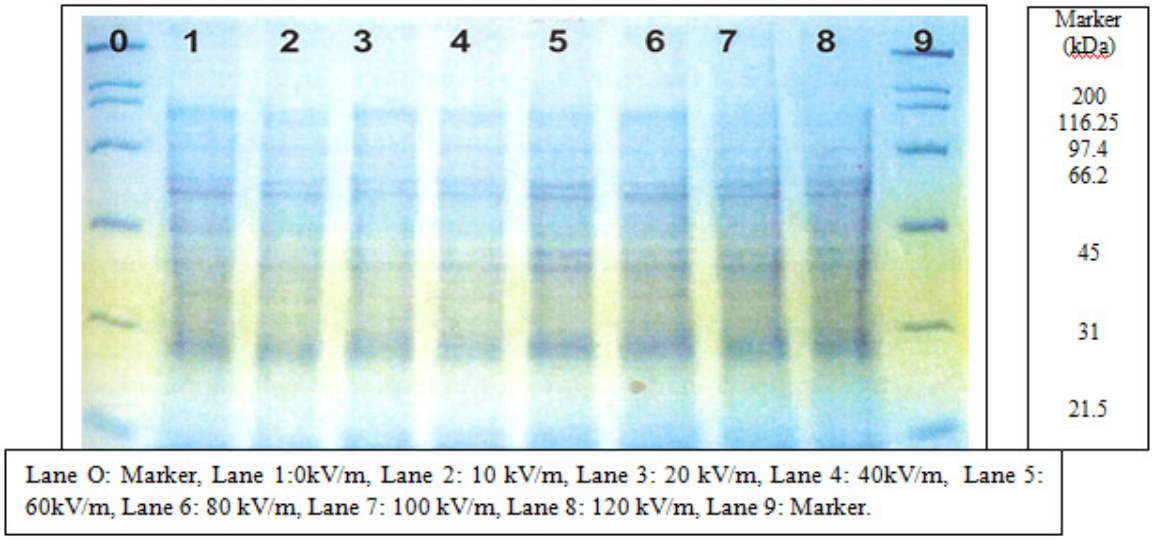


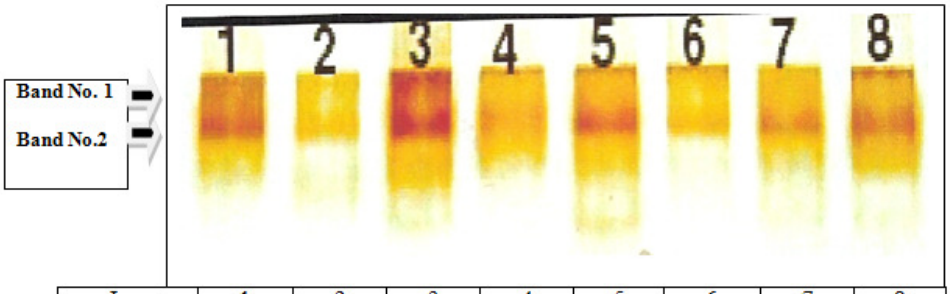
Fig 2. Effects of electric field on the 10% SDS PAGE protein banding profile of 12 weeks old *Oncidium taka* PLBs.

treatments, some differences in the subsequent plantlets that grew from the electric field treated PLBs were obtained after six months. Figure 1 shows the effects of electric field treatment of 0, 10, 20, 40, 60, 80, 100 and 120 kV/m respectively on *Oncidium taka* PLBs after six months of culture. Magone (1996) reported that electric field causes small cellular changes in *in vitro* duckweed that becomes evident only after replication during cell division. Thus more prominent effects were observed after some time after the electric field treatment. Therefore, short-term observations on organism's response to EMF will often reflect EMF as a stress factor but a long term study could yield different conclusions due to the effects becoming more evident only at later times. Figure 1 showed the plantlets that grew from the untreated and electric field treated *Oncidium taka* PLBs. Comparatively to the plantlets that grew from the electric field treated PLBs, the untreated plantlets (Figure 1a) recorded less growth increment. However, in comparison with the control and the other electric field treatments, the PLBs treated with 10 kV/m (Figure 1b) seemed to experience stunted growth where short leaves and roots were observed. No sign of vertical roots or long leaves were observed as was found in the other electric field treatments. The figure showed that the 10 kV/m treated PLBs was still at the shooting stage while the other electric field treated PLBs have developed into plantlets with long leaves and roots. Although 10 kV/m may be the least electric field strength studied, the results obtained suggests that the 10 kV/m treatment had reduced the growth of the PLBs, similarly to the mean fresh weight of the 10 kV/m treated PLBs at 12 weeks after treatment where it was the least compared to the other treatments (Table 2). However, in comparison to the ambient electric field of less than 0.2 kV/m (Horton and Goldberg 1995), the 10 kV/m treatment was considerably higher than the naturally present electric field. The reduction in cell elongation observed were similar to reports by Rozema et al. (1999) reported that the exposure of pear shoots to ultraviolet blue, another type of non-ionizing radiation similar to electric field resulted in an increase in ethylene, which promotes radial growth and reduces cell elongation. Figure 1c showed the subsequent growth and morphology of the *in vitro Oncidium taka* plantlets which grew from 20 kV/m electric field treated PLBs. The result was significantly ($p< 0.05$) different from the untreated and 10 kV/m treatment as the electric field treatment on the PLBs had resulted in vigourously growth after six months of culture. Visually, thicker root were observed with the presence of both upward and downward growth and leaves were larger due to greater width than the untreated leaves. The strong plantlets, which stemmed from the 20 kV/m treated PLBs correlates with the fresh weight of the PLBs taken at 12 weeks after treatment (Table 2) where 20 kV/m resulted in the second highest fresh weight after treatment 40 kV/m. Such observation on increased growth was similar to

Table 3. Effect of electric field on the soluble protein and nitrogen content of *Oncidium taka* PLBs twelve weeks after treatment.

Treatment (kV/m)	Soluble Protein Content (mg/g fresh weight)	Soluble Nitrogen Content ($x\ 10^{-1}$ mmole/g fresh weight)
0	7.95a	0.21d
10	7.28b	0.24c
20	4.57d	0.19e
40	4.75d	0.18e
60	5.98c	0.25b
80	6.39c	0.19e
100	5.77c	0.26a
120	5.95c	0.15f

Means with the same letter in a column are significant different according to DMRT (p=0.05).



Lane	1	2	3	4	5	6	7	8
Treatment (kV/m)	10	20	40	60	0	80	100	120
Band No. 1	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Band No. 2	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16

Fig 3. Peroxidase enzyme activity bands at 12.5 % polyacrylamide gel and the R_f values *Oncidium taka* PLBs twelve weeks after electric field treatment.

Lucchesini et al. (1992) who found increased in shoot number, rooting percentage, plant length and weight *in vitro* propagated plantlets of *Prunus cerasifera* treated with pulsed electromagnetic field. Upward growing roots observed in tissue cultured plants are a type of growth deformities known as inverted geotropism according to Magone (1996) who found growth disturbances such as upward growing roots and left symmetry growing on *in vitro* duckweed (*Spirodela polyrhiza*) cultures exposed to electric field between 0.6 V/m to 2.6 V/m after 30 days of growth. This also indicates that small cellular changes from the electric field treatment became evident only after replication during cell division. Similarly in the case of the electric field treated *O. taka* PLBs, a rather different growth pattern were observed among the plantlets six months after the treatment. However, orchid is an epiphyte and the appearance of upward growing roots may not be entirely due to the electric field treatment. Figure 1d showed the *in vitro Oncidium taka* plantlets, which grew from the 40 kV/m electric field treated PLBs six months after treatment. The 40 kV/m electric field strength enhanced the expansion and elongation of leaves, thus, resulting in long and wide leaves. Based on the observation (Figure1), higher top-growth with higher number of leaves, leaves length and width were observed compared to the untreated. The vigorous and rapid growth of the plantlets from the PLBs six months after treatment was due to the growth rate of the PLBs, namely the fresh weight gain and dry weight which were the highest at the early stage of the growth. However, the root growth of the plantlets was not as vigorous as those treated with 20 kV/m in Figure 1c. Figure 1e showed that the subsequent plantlets that grew from the 60 kV/m treated PLBs had less top growth and only medium length leaves were observed. However, all three directions of root growths, namely downward, upward and lateral directions were observed. Figure 1f showed the resulting plantlets from the 80 kV/m electric field treated PLBs which had a higher growth compared to Figure 1e (60 kV/m). The plantlets observed had longer and wider leaves than the control (Figure 1a) and those treated with 10 kV/m (Figure 1b) and 60 kV/m (Figure 1e) respectively. The appearance of both upward and downward growing roots was also observed. Figure 1g showed the effects of 100 kV/m electric field treatment on the growth of PLBs after 6 months of culture. The plantlet that grew from the high electric field treated PLBs developed some deformities where short leaf length and internode with thick diameter symbolizing some dwarf-like appearance were observed. However, the growth of the roots was not disrupted. Furthermore, the appearance of many upward growing roots from all around the plantlets was observed with more upward than downward growing roots. Figure 7h showed the subsequent growth of the *Oncidium taka* PLBs six months after the electric field treatment with 120 kV/m and produced vigorous root growth in the plantlets. Observation shows numerous upward and downward growing roots and the high growth rate of the downward growing roots was found to have pushed the base of the plantlet above the solid growth media, thus causing it to be slightly suspended in the air. Goldsworthy and Rathore (1985) suggest that cell uses the orientation of electric field to control its structure and direction of growth. Similar to the principle of electrophoresis which is based on the fact that ions or charged compounds migrate when placed in an

Table 4. Effect of electric field on the peroxidase enzyme activity of *oncicdium taka* PLBs twelve weeks after treatment.

Treatment (kV/m)	Total Enzym Activity (Unit/mL)	Specific Enzym Activity (Unit/mg protein)
0	294c	1859d
10	496a	3449b
20	177d	2428c
40	216d	2277c
60	421b	3517b
80	490a	4101a
100	457ab	3707b
120	289c	2430c

Means with the same letter in a column are insignificantly different according to DMRT (p = 0.05).

Table 5. Effects of electric field on the chlorophyll and carotenoid content of *Oncidium taka* PLBs twelve weeks after treatment.

Treatment (kV/m)	Chlorophyll a (µg/mL)	Chlorophyll b (µg/mL)	Chlorophyll a : b	Total Chlorophyll (mg/g fresh weight)	Carotenoid (mg/g fresh weight)
0	8.13 c	3.74b	2.17d	0.177c	0.051b
10	7.92e	3.45e	2.29a	0.170e	0.033e
20	8.13c	3.63c	2.24b	0.176d	0.047cd
40	12.02a	5.61a	2.14e	0.263a	0.068a
60	7.71f	3.54d	2.18d	0.168f	0.047cd
80	78.26b	3.73b	2.22c	0.179b	0.049bc
100	7.20g	3.32f	2.17d	0.157g	0.050b
120	8.07d	3.73b	2.17d	0.176d	0.046d

Means with the same letter in a column are insignificantly different according to DMRT (p = 0.05).

electric field; the stimulated electric field result in the electrophoresis movement of charged materials and vesicles containing the necessary substrates towards the sites of polar growth. Therefore, this may explain the accelerated root growth of the plantlets, which may be due to the migration of ions to the root area and thus stimulating growth. Goldsworthy and Rathore (1985) added that the applied electrical current might also cause the electrophoretic segregation of membrane-bound proteins and enzymes into bands along the electrical axes of the cell resulting in metabolic gradients within the cell and structural polarity. Moulder (1996) suggested that electromagnetic field influences biological systems via a transduction site on the membrane that converts electromagnetic energy into a chemical chain of reactions. Electrochemical events associated with the binding of molecules at receptor sites are stimulated and the surface events are amplified and signaled to the cell interior by coupling with intercellular enzyme systems. According to Bulychev and Vredenberg (1999), electric field affects the membrane potential, which controls the rates and direction of electron transport, ion fluxes and membrane enzyme activities such as H^+-ATPases. Generally, a cell's interior has a different ionic composition from the surroundings. This ionic imbalance creates a potential gradient at the membrane to allow entry of nutrients, and prevent the loss of metabolites (Goldsworthy 1987). The presence of the electric field may interrupt with the original potential gradient leading to growth stimulation or disruption as was observed in the PLBs growth. Although there were some physical differences observed between the *O. taka* PLBs six months after the electric filed treatment, there was no drastic morphological changes or pigmentation observed. The different observed seemed more likely to be caused by the different initial growth rate of the plantlets caused by the electric field treatments. However, since all the *O. taka in vitro* cultures were from the same source of plant and age and were exposed to the same growth conditions and period of subculture, a homogenous growth pattern is expected, therefore, the variation in growth rate and pattern of the plantlets observed were due to the electrical field treatment. Furthermore, the theory that plant cultures respond differently at different level of irradiation or ionizing radiation can be applied when using non-ionizing radiation such as electric field where it has been observed that the electric field effects on the *O. taka* cultures were not linear to the electric field strength and no clear relationship could be ascertained. Therefore, the results suggest that the certain optimum electric field strength were capable of inducing or disrupting growth.

Soluble protein and soluble nitrogen content

Table 3 showed the protein and soluble nitrogen content of the twelve weeks old PLBs after treatment with the electric field. It was found that all the electric field treated PLBs had lower protein content than the control with 20 and 40 kV/m electric field treated PLBs having the least as oppose to their growth response obtain in Table 1. The result obtained from Table 3 indicated that the high fresh weight obtained from the two treatments were due to high moisture content in the PLBs and not the protein or nitrogen solutes. Therefore, the resulting low protein and soluble nitrogen content in PLBs may due to the high utilization of soluble nitrogen or protein to increase growth and shoot formation. The low level amino nitrogen obtained may also be due to continuous transport of amino acids to surrounding active tissues. The decrease in ethanol-soluble nitrogen reflects the decrease in the total nitrogen suggesting that the treatment increased the mobilization of nitrogen to younger of parts of the plant (Munns et al., 1979). Furthermore, the protein content of the 10 kV/m treated PLBs were the highest compares to the other electric field treated PLBs as shown in table 3. This may be due to the low utilization of protein for plant growth and to develop structure such as leaves and roots. Perhaps the

electric field treatment may have retarded the mechanism involve in utilizing protein for plant growth and organs development. Munns et al. (1979) added that although decrease in protein content is usually found in stressed plant, net protein hydrolysis may not occur, and the plant may resume active growth promptly when stress is relieved. This may be the case here where the low protein content of the 40 kV/m treated PLBs did not result in growth disruption. Another reason pointed out by Dastur et. al (1963) was during high growth rate and maximum production of carbohydrate, high photosynthetic activity are linked with decreased protein formation due to the accumulation of utilization carbohydrates synthesized. Mansour (2000) reported that there are many possible factors which may explain the increased accumulation of amino acid in stressed plants, such as protein degradation, inhibition of protein synthesis, decrease in amino acid and amide export. Stress-induced protein degradation provides substrates for energy metabolism and amino acids for synthesis of new stress-induced proteins for survival and growth under the modified conditions. The highest soluble nitrogen content was found in the PLBs treated with 100 kV/m at 23% higher than the control treatment. The increase in soluble nitrogen content was as reported by Hasegawa et al. (1986) who found increased free amino acids during cell adaptation in salt-stressed *in vitro* cultures. However, the protein content of 100 kV/m treated PLBs was much lower compare to the control. This suggests that the high electric filed 100kV/m may be gave disrupted the formation of protein from the free nitrogenous compounds available. At a higher strength of electric field, 120 kV/m, the PLBs had the lowest soluble nitrogen content of 0.15 x 10^{-1}mmole/g fresh weight, which are 31% lower then the control. This suggest that the high electric filed strength may have hindered the production or accumulation of nitrogenous compounds or perhaps the low soluble nitrogen content was due to the high turnover rate of nitrogenous compound to other compounds in support of the increased growth of the PLBs as shown in the resulting plantlets in Figure 1h. The influence of the electric field on the nitrogenous compounds and protein was as reported by Bulychev and Vredenberg (1999) who states the electrical field affects the membrane potential and changes in the membrane potential and permeability will influence other physiological processes such as nitrogen metabolism and the action of stress factors on growth and development. The increase and decrease of protein content in electric treated PLBs signifies that protein were involved in plant's reaction with electric field and the intersection was different and dependent on the electric field strength.

Peroxidase enzyme activity

Table 4 showed that all the electric field treated PLBs resulted in significantly ($p < 0.05$) higher specific peroxidase enzyme activity compared to the control. This indicates that the presence of the electrical field pose as an environmental stimulant resulting in stress response. However, the specific peroxide enzyme activities did not increase with increasing electric field strength, thus indicating that the level of stress response was not linear to the electric field strength. Hadrami and Baaziz (1995) reported that peroxidase is also known to be implicated in cell wall rigidification and cellular differentiation. Therefore, the increase in the peroxidase activity may signify increased cellular differentiation. However, the enzyme activities obtained may not be the same at all stages of development. The difference in the enzyme activity may be an adaptive process of the PLBs to electric field. The significant ($p < 0.05$) increase in the peroxidase enzyme activity in response to electric field suggest the active participation of the enzyme scavenging free radicals. The increased activities of hydrogen peroxide scavenging enzyme give a circumstantial evidence of enhanced generation of superoxide radicals and hydrogen peroxide in the plant tissues. The highest peroxidase enzyme activity was found in PLBs treated with 80 kV/m with 4101 unit/mg protein followed by 100 kV/m, 60 kV/m with 3707, 3517, and 3449 unit/mg protein respectively. The highest growing PLBs treated with 40 kV/m had a significantly ($p < 0.05$) higher peroxidase enzyme activity than the control by 18%, thus suggesting that the stress response induced by the electric field did not result in reduced growth. Polle and Pell (1999) found that the plant with high peroxidase enzyme activity have higher anti-oxidative capacity where they are better protected from oxidative damage. Therefore, plants that are acclimated to environmental stresses usually have elevated anti-oxidative enzyme. In the case of salt-stressed plant, Sreenivasulu et al. (1999) reported that the increase in the total peroxidase activity of salt-adapted cells reflects the changes in the mechanical properties of the cell wall. In response to the electric field, the PLBs have increased their peroxidase enzyme activity to adapt and protect themselves from any possible or further damage.

Total chlorophyll and carotenoid content

Table 5 showed the significant ($p < 0.05$) increase in the total chlorophyll and carotenoid content of PLBs treated with 40 kV/m with 0.263 and 0.068 mg/g fresh weight, respectively, compare to the control 0.177 and 0.051 mg/g fresh weight. The total chlorophyll and carotenoid content had an increment of 48.6% and 33.3 % compared to the control (Table 5). The increase in the photosynthetic pigment content of the PLBs were correlated with the growth where the highest mean fresh weight was observed using electric field treatment of 40 kV/m. This suggest that higher growth of the plantlets from the 40 kV/m treated PLBs may be due to the high chlorophyll content in the PLBs of 12 weeks after treatment, which were the highest compared to the other treatments. This suggests a possibility of the 40 kV/m electric field stimulating the increase in the chlorophyll content. The high carotenoid content in the 40 kV/m treated PLBs may also contribute to the increased growth of plantlets by providing protection against any photo-oxidation. Besides the 40 kV/m electric field treatment, 80 kV/m also resulted in higher chlorophyll content than the control with a 1.1% increase with 0.179 mg/g fresh weight. Table 5 also showed that the 100 kV/m electric field treatment resulted in the lowest chlorophyll content in the PLBs twelve weeks after treatment. Therefore, the reduced leaf growth in the subsequent plantlets may be due to the low chlorophyll content in the PLBs or vice versa. Shimada et al. (1995) linked the relationship between leaf chlorophyll content and photosynthesis with changes in the former affecting plant's productivity via photosynthesis. Low pigment content in 60 and 100 kV/m treatments could be an adaptive feature to reduce photodynamic destruction of the chloroplasts during

high irradiance and temperature. Kulandaivelu et al. (1989) reported that the down regulation of photosynthesis happens at the chloroplast level where low chlorophyll content usually correlates with the ultrastructure of the chloroplast where less number of thylakoids or poorly developed grana was reported. Although treatment 60 and 100 kV/m had low protein and chlorophyll content (Table 5), it had high soluble nitrogen content (Table 3). Martin and Thimann (1972) reported as decrease in chlorophyll and protein content with an increasing in α-amino nitrogen content in senescing leaves where the chlorophyll loss was accompanied by protein loss and a concomitant rise in the nitrogen level. Another factor for the changes in the chlorophyll content may be due to the structural changes on the lipid-protein domain of chloroplast membrane and cages in the proton channel and ATPase caused by the electric field as was reported by Rosemberg et al. (1994) where 40 kV/m may have caused some structural changes leading to increased chlorophyll content (Table 5). The result obtained suggest that electric field treatment of 40 kV/m was capable of stimulating the production of higher photosynthesis pigments leading to an increased photosynthesis, which increase growth in the *Oncidium taka* PLBs. According to Nedunchezhian and Kulandaivelu *(*1995), maximum pigment content occurs in plants during their generative phase and high chlorophyll content increases absorption of solar energy while high carotenoid content increases their protective role against photodynamic destructive.

SDS PAGE protein profile

According to the Cooke (1984) the electrophoretic analysis of the total soluble protein provides a good indicator of plant's response to external stimuli than enzyme since enzyme are single gene products, which may not be detected, in some particular plant. Protein and peroxidase enzyme analysis banding pattern analyses from leaf extracts indicate the possible genetic modification due to the electric field treatment. Figure 2 showed the SDS PAGE protein banding profile of *Oncidium taka* PLBs treated with various electric field strengths. Although some morphological variations were observed among the *Oncidium taka* plantlets six month after the electric field treatment, there was no drastic difference quantitatively detected in the protein electrophoretic profile. Matsumoto and Tamaguchi (1989) reported that in addition to the vigorous root formation in banana PLBs after treating with gamma ray, there was also a disappearance of the a 73kDa protein and the appearance of the new 52 kDa protein band in the gamma ray irradiated banana PLBs. However, in the case of the *Oncidium taka* PLBs, the electric treatment was non-ionizing radiation, therefore, no drastic morphological changes and no quantitative difference were observed in the protein banding profile (Figure 2). No novel band of was detected in the between the electrical field treated cultures and the control. However, there were some difference in the intensities of the banding profile where lane 3 (20 kV/m) and lane 4 (40 kV/m) had the least intensity while line 5 (60 kV/m), 6 (80 kV/m) 7 (100 kV/m) and 8 (120 kV/m) had similarly higher intensities. The protein banding pattern of the untreated PLBs at lane 1 also had higher intensities compare to the other lanes. Since the same amount of protein was applied to each line, beside their qualitative differences, the increase in the abundance of polypeptides expression could be the marker for physiological changes. Yuffa et al. (1994) stated that changes in the protein pattern usually correlate with histological difference in calli, therefore, in can be suggested the histological studies can be done in any further attempt to understand the effect of high electric field on plant cells. The protein banding profile intensities correlated with the total protein content of the PLBs where the lower protein content were from treatment 20 and 40 kV/m and other electric field treatment yield higher protein content. According to Begger et al. (1985), cells and tissue cultures contains relatively low content of soluble protein proves to be rather to difficult to obtained a clear protein banding pattern on the *Oncidium taka* PLBs. The protein banding pattern obtained was low in resolution due to the low protein content of the *in vitro* PLBs cultures which consist mostly of water.

Peroxidase enzyme activities band

The peroxidase enzyme bands staining of the electric field treated *Oncidium taka* PLBs revealed a total of two bands at R_f 0.09 and 0.23in the untreated PLBs (lane 5), and those treated with 40, 100 and 120 kV/m at lane 3, 7 and 8 respectively (Fig 3).

Sunflower cells synthesized new polypeptides including heat shock proteins (hsps) in response to heat shock (40^{o}C for 3 hours), which the synthesis of other polypeptides was suppressed which leads to the rapid decline of other protein production. The peroxidase activity banding pattern intensities could not be related to the peroxidase specific enzyme activities as shown in Table 4 since treatment 80 kV/ (lane 6) which has the highest specific enzyme activities resulted in the last enzyme activity bands staining. The highest enzyme bands staining were observed in lane 3, which correlate with the 40 kV/m treatment, followed by the 10 kV/m treatment (lane 1) and 0 kV/m (lane 5) treatment. The biological significance of the intensification of peroxidase band from 40 kV/m and 10 kV/m and the absence of peroxidase activity band at R_f 0.23 as observed in the PLBs treated with high electric field of 10, 20, 60, and 80 kV/m is not clear in this stage, but the increase in abundance and missing bands indicate the cell's response to the electromagnetic filed via changes in the peroxidase activity enzyme banding pattern.The increase in the relative amount of polypeptides or peroxidase enzyme activity bands in PLBs treated with electric field lends support to the suggestion that the observed response may be related in some way to electric field-related mechanisms or mechanism involving charged ion in plant cells. However, no linear relationship could be established between the peroxidase enzyme activity banding pattern and the increasing electric filed, similarly to the effect of using gamma ray, an ionizing radiation of orchid hybrid *Mokara* Chark Kuan cultures were similar pattern of DNA bands detected on the Southern blot found in 0, 20, and 80 Gy were different from treatments with 25, 40, and 50 Gy (Adrain 1999). Perhaps in the case of both ionizing and nor-ionizing radiation, the effects on plants are exclusive to certain irradiation strength only.

Glutamate oxaloacetate transaminase enzyme activity bands

Glutamate oxaloacetate transaminase (GOT) enzyme activity bands from *Oncidium taka* PLBs were not clear with low

resolution due to the relatively low protein content of PLBs which consisted mostly of water and the low visibility and high sensitivity of glutamate oxaloacetate transaminase enzyme activity bands in general.

Materials and methods

Oncidium taka protocorm-like bodies (PLBs)

Orchid hybrid (*Oncidium taka*) protocorm-like bodies (PLBs) were initiated from shoot tips and maintained in half strength Murashige and Skoog (1962) liquid media at pH 5.7 supplemented with 3% sucrose. The mineral component of the growth medium is a balanced mixture of all the macronutrients and micronutrients needed for plant growth, and the concentrations used have been optimized for the growth of *Oncidium taka* PLBs. The cultures were maintained at 25ºC in a continuous photoperiod environment throughout the experimental study.

Electric field source and treatment

Ten medium sized PLBs measuring approximately 1.0 cm in diameter were placed on half strength solid MS media and exposed to electric field created through the generation of high voltage from an alternating current (a.c.) transformer. The apparatus for creating high electrical field were similar to the one used by Bachman and Reichmanis (1973a), which consists of an upper and lower disc electrode where vertical uniform electrical field was created in the space between the two disc electrodes known as the inter-electrode space. The distance between the electrodes was adjusted to obtain the desired electric field strength in kV/m. The PLBs were exposed to eight electric field strengths of 0, 10, 20, 40, 60, 80, 100 and 120 kV/m. for one hour each. Control plants underwent identical treatment with 0 kV/m. Each treatment consists of five replicates. After the electric field treatments, the PLBs were left for three days, after which they were transferred onto fresh solid half strength MS media for a regeneration into plantlets and the increase in fresh weight were measured three weekly for a period of twelve weeks. After the period of twelve weeks, the PLBs were sub-cultured by transferring actively growing portions in fresh media every six weeks. A live and actively growing protocorm is green in colour while the dead ones are brown.

Experimental designs

The experimental design used was a completely randomized design with eight treatments and five replicates each. A controlled and homogenous condition was employed throughout the study.

Growth studies

Growth parameters such as fresh weight gain were monitored to determine the effects of electric field on the growth and development of the PLBs. After twelve weeks of culture, the percentage of growth was measured as the increase in fresh weight, dry weight and moisture content. The PLBs were harvested for the biochemical analysis after twelve weeks of culture.

Fresh weight determination

Initial fresh weight of the PLBs were taken before the electric field treatment and the fresh weight of the PLBs clumps or plantlets which have multiplied or regenerated from the initial PLB were measured every three weeks up till twelve weeks after treatment. Fresh weight was measured by drying the PLBs on Whatman filter paper No.1 before weighing on a four decimal place electronic balance. The mean fresh weight gain of the cultures was determined every three weekly as described by Lutts and Guerrier (1995) as follows:
Mean Fresh Weight Gain (g) = Current Fresh Weight – Initial Fresh Weight at 0 week
Growth enhancement or inhibition was determined after twelve weeks and was expressed as percent increase or decrease in fresh weight according to the equation by Jain et al. (1990):

Percent Increase in Weight = Final weight – Initial weight X 100 Initial weight

Dry weight determination

After twelve weeks of growth, the PLBs were harvested and placed on two pieces of Whatman filter paper No.1 on Petri dishes before drying at 70ºC in a ventilated oven for 24 hours. The oven-dried PLBs were cooled to room temperature in a desiccating jar containing silica gel and weighed. The same procedure was repeated until a constant dry weight was obtained. All the weighing procedures were done using a four decimal electronic balance. The growth of the treated cultures in fresh weight was determined after twelve weeks according to the equation by Jain et al. (1990). Moisture content (%) is expressed based on the difference between the fresh and oven-dried weight over fresh weight before drying.

Soluble protein content determination

After twelve weeks of culture, the PLBs of the same batch and age were harvested and pulverized with liquid nitrogen before homogenizing with protein buffer using a chilled pestle and mortar at a ratio of 1:3 (g sample: mL extraction buffer). The homogenate was centrifuged at 12,000rpm for 20 minutes at 4^0C. The pellet was discarded and crude soluble fraction was subjected to ammonium sulfate precipitation.

Assay for soluble protein content
Soluble protein content of the PLBs obtained was determined according to the Coomassie Blue dye-binding method of Bradford (1976).

Soluble nitrogen content determination

After twelve weeks of culture, the PLBs of the same batch and age were harvested. Soluble nitrogen content of the PLBs obtained was determined according to the Cadavid and Paladini (1964).

Peroxidase enzyme activity determination

The enzyme extract used for the determination of peroxidase enzyme activity was prepared following the protein extraction method.

Assay for POX enzyme activity

Peroxidase activity (POX) (EC 1.11.1.7) was measured by monitoring the formation of tetraguaiacol (extinction coefficient 26.6 mM-1cm-1) from guaiacol using the method of Chance and Maehly (1955). The POX reaction solution (3 ml) contained 0.5 mM phosphate buffer (pH 6.1), 16 mM guaiacol, 2mM H_2O_2 and 20 ml enzyme extract. Changes in absorbance of the reaction solution at 470 nm were determined every 20s. One unit POX was defined as an absorbance of 0.01 units per min.

Total chlorophyll and carotenoid determination

After twelve weeks of culture, the PLBs of the same batch and age were harvested, washed with sterile distilled water and wiped dry. Total chlorophyll consisting of chlorophyll a and b, and carotenoid, which consist of carotene and xanthophylls, were determined following the method of Harborne (1984).

Statistical analysis

All the data obtained for biochemical analyses were the mean of three readings per replicate per treatment. Each biochemical experiments were repeated thrice with independent extractions to verify the results obtained. Growth parameters taken from the *in vitro O. taka* cultures were the mean of five readings per replicate with four replicates per treatment. The data obtained were subjected to analysis of variance (ANOVA) and means between treatments were compared using Duncan Multiple Range Test (DMRT) at the significance level of 0.05 using PC-SAS System version 6.12.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS PAGE)

The SDS-PAGE protein banding pattern of were compared using the Bio-Rad Mini PROTEAN 3.

Enzyme activity banding pattern

Enzyme activity banding pattern were done using native polyacrylamide gel electrophoresis (PAGE). Enzyme extracts was prepared according to the soluble protein content method and 10 µg proteins were loaded onto the gel wells. The native PAGE was run at 4ºC for approximately four hours.

Peroxidase enzyme activity bands

After the non-denaturing polyacrylamide gel electrophoresis was completed, the gels were stained for peroxidase enzyme activity bands.

Glutamate oxaloacetate transaminase (GOT) enzyme activity bands

Similarity, native electrophoresis gels were stained for GOT.

Protein and enzyme activity banding evaluation

The protein-banding and enzyme activity banding patterns were observed on a light box and photographed. The protein banding and enzyme activity banding patterns were compared and assessed.

Conclusion

Therefore, the result obtained from this study reveals the potential of using 40 kV/m electric field strength to stimulate the production of higher photosynthetic pigment namely chlorophyll and increasing of *Oncidium taka* PLBs. The finding opens the way for more work to optimise and manipulate the use of electric field as a tool to increase plant yield via tissue culture method. However, the result also showed the difficulties in obtaining and establishing a clear relationship between the influencing electric field and the protein, nitrogen content or peroxidase enzyme activity. Nevertheless, this study has found that the increase in plant growth as reported by many other studies are not related to any changes in the protein banding profile as no drastic difference were obtained. The differences in the peroxidase enzyme activity banding patterns may be related to the growth differences of the PLBs resulting from the electric field treatment.

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