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Effect of some phytohormones on growth characteristics of *Chlorella sorokiniana* IAM-C212 under photoautotrophic conditions

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The effects of some phytohormones [indole acetic acid (IAA), indole butyric acid (IBA), gibelleric acid (GA_3), and kinetin] on cell dry weight, cell number, cell size, protein and chlorophyll contents of *Chlorella sorokiniana* IAM-C212 were investigated under photoautotrophic conditions. Treatment with IAA (15 mg/l) and IBA (15 mg/l) resulted in significantly higher dry cell weight and cell number than the control ($P < 0.05$) but there were no significant effects of GA_3 and kinetin on cell growth as expressed by dry cell concentration (g/l) and cell population (cells/ml). Treatment with IAA at a concentration of 10 or 15 mg/l gave the highest cell dry concentration of 4.68 g/l after eight days of cultivation, which is more than 9 times higher than the value obtained in the control culture (without phytohormone). The optimum concentration of each of the phytohormones for *C. sorokiniana* cell enlargement was 20 mg/l. At this concentration, the average cell sizes were 81.07, 78.67, 78.07, 66.90 and 68.1 µm for GA_3, kinetin, IAA, IBA and control, respectively. Addition of 15 mg/l of IAA or GA_3 to the culture resulted in significantly higher extractable chlorophyll contents than the control ($P < 0.05$) but the effects of IBA and kinetin were not significant ($P > 0.05$). The protein contents of the cells cultivated with 20 or 10 mg/l of GA_3, 15 mg/l of kinetin, and 15 mg/l of IBA or IAA were 46.64, 45.83 and 45.81%, respectively. In the control experiment, the protein content was 43.38% after eight days of cultivation, showing that treatment with these phytohormones had no significant effect on the protein contents of the cells ($P > 0.05$). Combination of IBA and GA_3 exhibited synergistic effect on growth and productivity of *C. sorokiniana* but there was no synergistic effect when IAA was combined with either GA_3 or kinetin.

Key words: Phytohormones, *Chlorella sorokiniana*, cell growth, cell size, protein content, chlorophyll content.

INTRODUCTION

Microalgae biomass has numerous applications which include its use as a nutritional supplement for humans and animals to improve immune response and fertility, sources of biofuels, cosmetic supplement, natural food

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Abbreviations: IAA, Indole acetic acid; **IBA,** indole butyric acid; **GA_3,** gibelleric acid.

colourants, sources of high-value functional ingredients such as polyunsaturated fatty acids, ω-3 fatty acids and pigments (Griffiths and Harrison, 2009; Heydarizadeh et al., 2013; Salama et al., 2014). Many of these applications have commercial values but their production costs are still high due to low growth rates of many microalgae species. Many of these applications of microalgae demand the use of monocultures and controlled cultivation systems. These requirements have led to increased emphasis on development of cultivation strategies for efficient and cost-effective production of algae biomass. A lot of work has been done on photobioreactor design and optimization for efficient cultivation of microalgae but the reported productivities are still low due to some technical problems with light supply and distribution inside such reactors (Ogbonna and Tanaka, 2000; Oliver et al., 2013; Park et al., 2013). Furthermore, efforts to develop strains with high growth rates and productivity through genetic engineering approach have not yielded the desired results (Potvin and Zhang, 2010; Brennan et al., 2012).

Phytohormones have been used to improve productivities in higher plants since the 1930s (Piotrowska et al., 2008). Auxins (Indoleacetic acid, Phenylacetic acid, Indolebutyric acid and Naphthalene acetic acid) are a class of phytohormones that primarily increase growth in plants (Woodward and Bartel, 2005; Salama et al., 2014). Indole acetic acid (IAA) is a natural auxin and one of its primary effects is activation of the Plasmalemmal H^+ - ATPase involved in the process of growth by elongation (Tarakhovaskaya et al, 2007). Auxins accomplish these tasks by increasing cell wall plasticity, increasing water intake, altering respiratory patterns and altering nucleic acid metabolism (Woodward and Bartel, 2005). Cytokinins (trans-zeatin and kinetin) are plant growth substances which play a role in senescence and chloroplast development, primarily by promoting cell division (Tarakhovskaya et al., 2007). Endogenous Cytokinin-like activity has been documented in various microalgae (Stirk et al., 2002; Filomena et al., 2013). Effects of cytokinins have been determined in higher plants by exogenous addition of cytokinins. Most of the data on the effects of exogenous cytokinins on algal growth and development are on the members of the division Rhodophyta. Cytokinins (alone or in combination with auxins) were shown to accelerate red algal growth in the culture and in some cases, facilitate callus formation (Yokoya et al., 1999; Bajguz and Piotrowska-Niczyporuk, 2013). Thus, cytokinins, as cell-division promoting substances, may induce a faster growth rate in algae cells as they do in higher plant species. This fact, along with the detection of cytokinin-like activity in algae cells, is encouraging and highlights the potential for these substances to promote bioactive molecules and biofuel productions from algae. Gibberellins are diterpenoid acids that affect many areas of plant growth. They promote stem elongation, fruit generation and seed germination (Nakajima et al., 2006).

Application of Gibberellins caused cells to increase in size (Gonai et al., 2004). Little evidence for endogenous gibberellins activity has been observed in green algae. Although increased growth in response to gibberellins has been documented in some algae (Jennings, 1968; Joseph and Chennubhotla, 1999), there is scarce evidence for its actions beyond those in higher plants. Microalgae share some physiological similarities with higher plants. Although contemporary research on phytohormone physiological actions remain almost completely focused on the higher plants, there are few studies devoted to auxins and other classes of phytohormones in green algae such as *Chlorella* and *Scenedesmus*, and in Cyanobacteria such as *Spirulina* (*Arthrospira*) species (Czerpak et al., 1994; 1999). Studies with *Chlorella* species showed that the use of phytohormones have considerable stimulating effects on algal growth and productivity (Czerpak et al., 1994; 1999). Although, all the phytohormones used in their studies had considerable stimulating effect on *Chlorella pyrenoidosa*, it is imperative to determine whether such effect is common to all species of *Chlorella* and to determine their effects on other growth characteristics. To the best of our knowledge, this is the first report on the effects of these various phytohormones on the growth characteristics of *Chlorella sorokiniana* IAM-C212. The specific objectives of this study are: (i) to determine the optimal concentrations of the selected phytohormones for cell biomass (dry cell weight, cell number and cell size) as well as the chlorophyll and protein contents of the cells, and (ii) to evaluate synergistic effects of combinations of the selected phytohormones on the growth characteristics of *C. sorokiniana* IAM-C212.

Microalgae growth involves chlorophyll development, cell division and cell enlargement. Thus, representatives of classes of phytohormones that have been reported to stimulate these processes in higher plants, namely auxins (cell enlargement), cytokinines (chloroplast development and cell division), and gibberellins (cell elongation and enlargement) were selected for this study.

MATERIALS AND METHODS

Strain of microalgae and sub-cultures

Axenic strain of *C. sorokiniana* IAM-C212 used in this study was obtained from the Culture Collection Centre, University of Tokyo, Japan and maintained in basal growth medium as modified by Ogbonna et al. (1997). The basal growth medium was composed of (g/l): urea, 1.2; KH_2PO_4, 0.3; $MgSO_4.7H_2O$, 0.3; $CaCl_2$, 0.02; sodium citrate, 0.05; Fe-solution, 0.16 ml; and A_5 solution, 0.8 ml. The Fe-solution was composed of 25 g of $FeSO_4.7H_2O$ and 33.5 g EDTA per liter of distilled water. A_5 solution was composed of (g/l): H_3BO_3, 2.86; $MnCl_2.4H_2O$, 1.81; $ZnSO_4.7H_2O$, 0.22; $CuSO_4.5H_2O$, and MoO_4, 0.015. The pH of the culture medium was adjusted to

6.5 ± 0.2 before inoculation. The culture was maintained in a temperature controlled growth chamber at 25 ± 2°C illuminated with six 12 watts energy saving bulbs fixed on two parallel wooden boxes each placed at a distance of 10 cm from the conical flasks containing the culture medium. The mean light intensity on the surface of the flasks was 90±5 µmol m^{-2} s^{-1}. The stock was sub-cultured every month.

Selection of the phytohormones

The four phyto-hormones used in this study were indoleacetic acid (IAA), indolebutyric acid (IBA), gibberellic acid (GA_3) and kinetin. The IAA, IBA, GA_3 and Kinetin were purchased from Wako Pure Chemical Industrial Ltd, Tokyo, Japan.

Phytohormone stock solutions

Twenty milligrams of each of the phytohormones was first dissolved in the appropriate solvent (GA_3 in 5.0 ml of de-ionized water, IAA and IBA in 0.5 ml of 95% ethanol, and kinetin in 0.1 N hydrochloric acid) and then made up to 200 ml with de-ionized water to obtain 100 mg/l which served as the stock solution. Desired concentrations: 5, 10, 15, and 20 mg/l were prepared from the stock solution.

Synergistic study

The effect of combined phytohormones on the growth characteristics of *C. sorokiniana* IAM-C212 was studied using the following phytohormone combinations: IBA combined with GA_3; and IAA combined with GA_3.

Cultivation method

Five hundred milliliter (500 ml) Erlenmeyer flasks containing 300 ml of the basal growth medium supplemented with various concentrations of phytohormones or combinations were inoculated with 15 ml of a 4 day old culture of *C. sorokiniana* containing 5.00 × 10^8 cells/ml and incubated statically but mixed manually three times daily for 8 days in a growth chamber illuminated by six-12 watts energy-saving bulbs fixed on two parallel rectangular wooden boxes. The mean light intensity on the surface of the flasks was 90 ± 5 µmol m^{-2} s^{-1} while the temperature was controlled at 30 ±2°C. A 10 ml of the culture broth was aseptically drawn on 48 hourly bases for assay.

Analyses

The dry cell weights were determined using triplicate samples of the growth culture solution. A 10 ml of algal culture was filtered through a pre-weighed Whatman No 1 filter paper after centrifuging at 3000 rpm for 15 min. The filter paper was washed with 5 ml of 0.1 N HCl to remove the precipitated salts and dried overnight at 80°C in an oven. Dried filter paper with biomass was cooled in a desicator and weighted again to estimate the final dry weight of the algae (Ogbonna et al., 1997). The cell number was measured on 48 hourly bases by counting the cell number using light microscope and Neubaur counting chamber. The cell size was measured using Moticam Images Plus 2.0 digital camera (Motic China Group Ltd) connected to a microscope and computer system. For determination of chlorophyll contents, a 10 ml of algal culture broth was centrifuged at 3000 rpm for 20 min, and the chlorophyll was extracted from the algae pellet using 4 ml of methanol (95%). The amount of chlorophyll extracted in the methanol was determined spectrophotometrically according to the method described by Ogbonna et al. (1997), using the following equation:

Chlorophyll (µg/ml^{-1}) = 25.5 (A650-A 750) + 4.0 (A 665 - A 750).

Here, A650, A665 and A750 are absorbance at 650, 665 and 750 nm, respectively. The values were converted to mg/g by dividing by the respective cell concentration in the sample.

The protein content was determined using 0.2 g of dry algal sample to estimate the nitrogen content of the biomass. The nitrogen contents were multiplied with the nitrogen - to - protein conversion factor of 6.25. The nitrogen content was measured according to AOAC method of analysis (2010). A 0.2 g weight of the dry biomass was added into a clean and dry digestion flask (Kjedhal flask). Selenium powder (0.05 g), Copper sulphate (0.5 g) and Sodium sulphate (2 g) were added. This was followed by the addition of 20 ml of concentrated H_2SO_4. The solution was swirled until it darkened and then heated in a fume cabinet until it became clear. The digested sample was diluted to 100 ml with distilled water and 5 ml taken for distillation. A 10 ml of 50% sodium hydroxide was added to 5 ml of the sample in a Markham apparatus and the solution allowed to distil over 10 ml of boric acid mixed indicator until the indicator turned light green. A 50 ml volume of the distillate was titrated against 25 ml of 0.01 N HCl until the first pink appearance occured. The percentage nitrogen was calculated.

Statistical analysis

All the data were subjected to one way analysis of variance (ANOVA). Where, there were significant effects, least significant difference (LSD) was used to separate the means. The results were expressed as mean ± standard error of the mean.

RESULTS AND DISCUSSION

Effect of phytohormones on dry cell weight of *C. sorokiniana*

Treatment with IAA, IBA and kinetin resulted in significantly higher biomass concentration than the control ($P < 0.05$) while treatment with GA_3 resulted in lower biomass concentration. IAA at a concentration of 10 mg/l gave the highest cell concentration of 4.685 g/l and productivity of 0.586 g/l/day as shown in Figures 1 and 2. This compares with the control (without phytohormone) which had an average biomass concentration of 0.489 g/l. Using Least Significant Difference, the effectiveness of the phytohormones in increasing the dry cell concentration of *C. sorokiniana* can be ranked as IAA (4.685 g/l) > IBA (1.664 g/l) > Kinetin (0.621 g/l) > GA_3 (0.471 g/l). The optimum concentration of each phytohormone was between 10 and 15 mg/l and the effectiveness of the phytohormone concentrations was ranked as (10 = 15 mg/l) > 20 > 5 mg/l. As shown in Figure 1, the effect of IAA was not significant in the first 3 days and the average cell concentration was 0.157 g/l compared with the 0.101 g/l

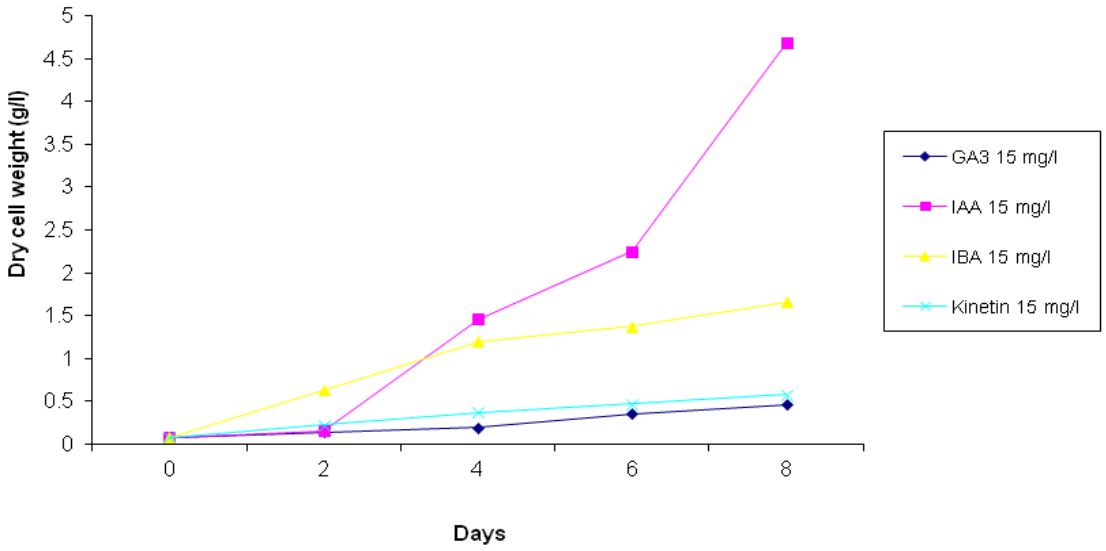

Figure 1. Effect of phytohormones on dry cell weight of *Chlorella sorokiniana* IAM C212.

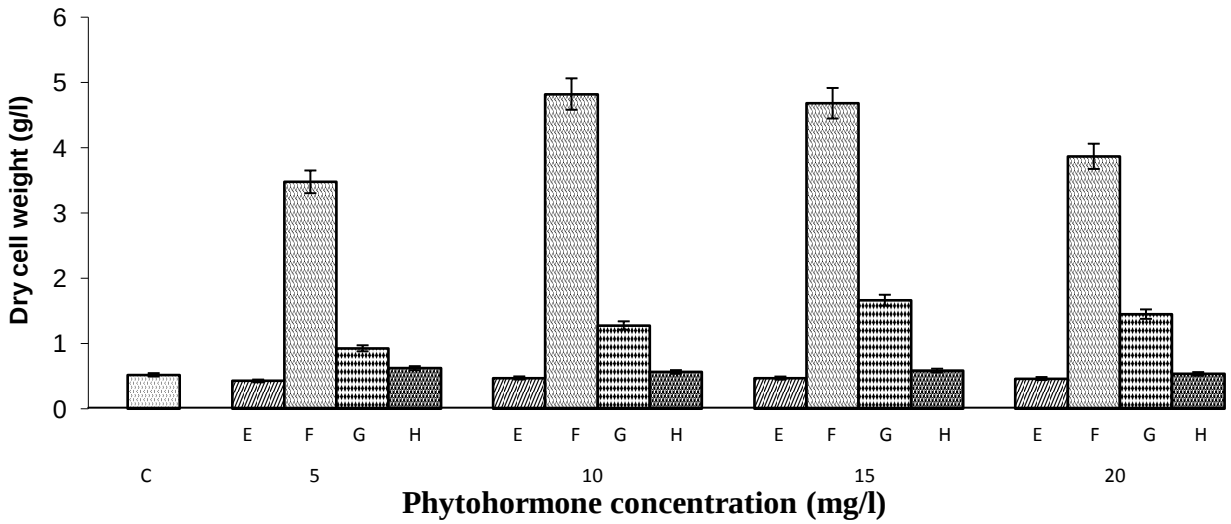

Figure 2. Effect of phytohormones on dry cell weight of *Chlorella sorokiniana* IAM C212. Symbols: C, Control; E, GA_3; F, IAA; G, IBA; H, kinetin.

obtained in the control culture during that period. In contrast, IBA increased the biomass productivity in the first 3 days, but maintained a gradual and steady increase thereafter, resulting in an 8 day average of 1.664 g/l compared with the 0.489 g/l obtained in the control experiment. These differences may be explained on the basis of nutrient transport into the cells. The algal cells tend to have a residual negative charge. At neutral or alkaline pH, organic acids such as IAA and IBA do not enter the cells due to their ionic forms (Dibb-Fuller and Morris, 1992). At acidic pH values, these compounds are non-ionized and can enter the negatively charged cells. IAA having lower molecular weight than IBA diffuses faster across the cell membrane of the algae cells. The cell will try to maintain its internal pH by neutralizing or expelling the protons but this will slow down the growth as it diverts energy from growth-related functions (Rayle and Cleland, 1992). This brings about increased lag phase. In other words, the length of the lag phase may reflect the time necessary for the algae cells to bring the external environment within their optimum growth range. Hunt et al. (2010) experimenting with Naphthaleneacetic

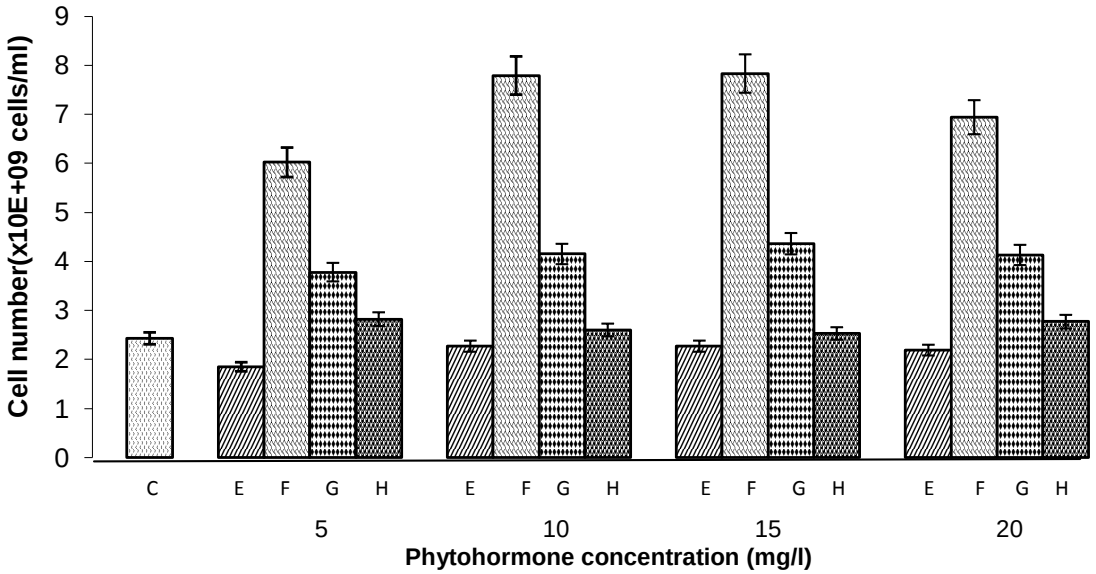

Figure 3. Effect of phytohormones on cell number of *Chlorella sorokiniana* IAM C212. Symbols: C, Control; E, GA_3; F, IAA; G, IBA; H, Kinetin.

acid (NAA) over a 10 day static culture observed that NAA did not enhance the growth of *C. sorokiniana* in the first 5 days of the cultivation but significantly enhanced growth and productivity of the algae cells thereafter. They opined that it could be as a result of longer lag and prolonged exponential phases of algae cells exposed to NAA. Since IAA and NAA belong to the auxin family, IAA may have elicited similar response from *C. sorokiniana* IAM-C212 in this experiment. Unlike NAA, it took a shorter period of time (3 days) for the algae cells to get acclimatized to IAA in this experiment.

Effect of phytohormones on *C. sorokiniana* cell number

The result of the effect of different concentrations of phytohormones on cell number of *C. sorokiniana* after 8 days of cultivation is shown in Figure 3. There were also significant effects of the phytohormones on cell number ($P < 0.05$). Amongst the phytohormones, IAA at a concentration of 15 mg/l gave the highest cell number with an average value of 7.83×10^9 cells/ml. This compares with the control (without phytohormone) which had an average value of 2.43×10^9 cells/ml. The effectiveness of the phytohormones on the cell number of *C. sorokiniana* can be ranked as IAA (7.83×10^9 cells/ml) > IBA (4.36×10^9) > Control (2.43×10^9 cells/ml) > Kinetin (2.27×10^9 cells/ml) > GA3 (2.19×10^9 cells/ml) and the effectiveness of the various concentrations was ranked as (15 =10 mg/l) > (20 = 5 mg/l). Contrary to its effects in higher plants (initiation of cell division), kinetin did not bring about an appreciable increase in cell number of the algae cells. Cytokinin signal transduction pathway begins with binding to a two-component receptor system, involving the cytokinin receptor, CR2 (Inoue et al., 2001). Along these pathways, regulatory proteins play a critical role in increasing and decreasing the cytokinin signal. The increased growth from cytokinins is a product of the activation of these regulators of the cell division cycle and differentiation (Sheen, 2001; Rióu-Khamlichi et al*.,* 1999). These regulatory proteins may be lacking or inactive in *C. sorokiniana* IAM C212.

Effect of phytohormones on cell size of *C. sorokiniana*

The result of the effect of different concentrations of phytohormones on cell size of *C. sorokiniana* after 8 days of cultivation is shown in Figure 4. The optimum concentration of phytohormones for *C. sorokiniana* cell enlargement was 20 mg/l for each of the phytohormones. At this concentration, the average values of the cell sizes were 81.07, 78.67, 78.07 and 66.90 µm for GA_3, Kinetin, IAA, and IBA, respectively. This compares with the control (without phytohormone) which had an average value of 68.43 µm. Treatment with GA_3, Kinetin and IAA significantly increased the cell size ($P < 0.05$) while there was no significant difference between the average sizes of the cells treated with IBA and those of the control. The effectiveness of the phytohormones in increasing the size

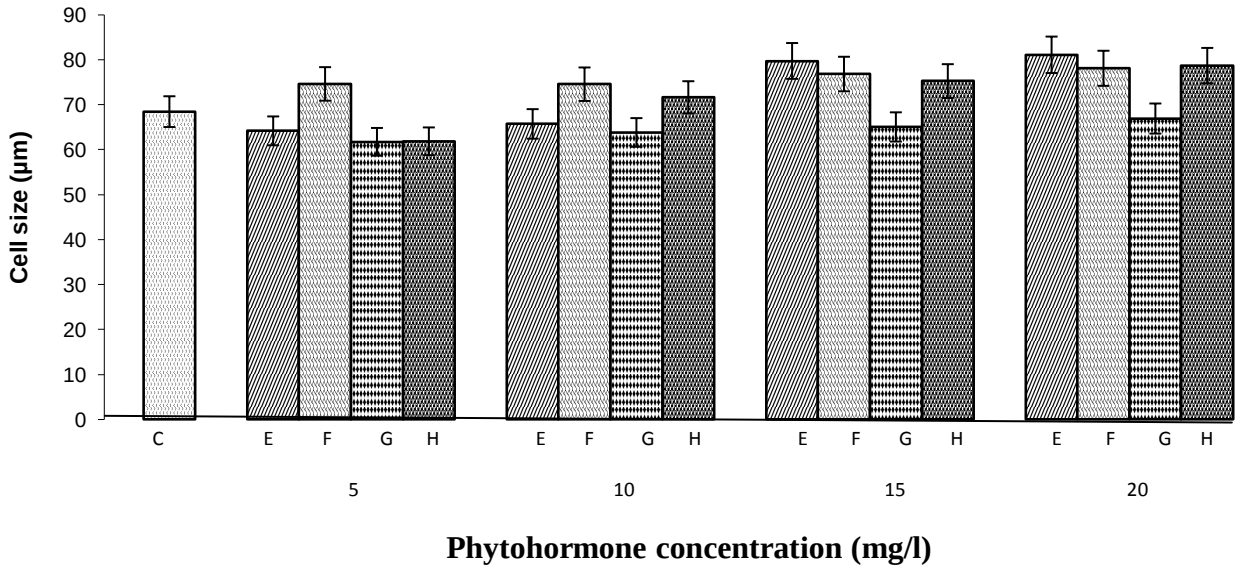

Figure 4. Effect of phytohormones on cell size of *Chlorella sorokiniana* IAM C212. Symbols: C, Control; E, GA_3; F, IAA; G, IBA; H, kinetin.

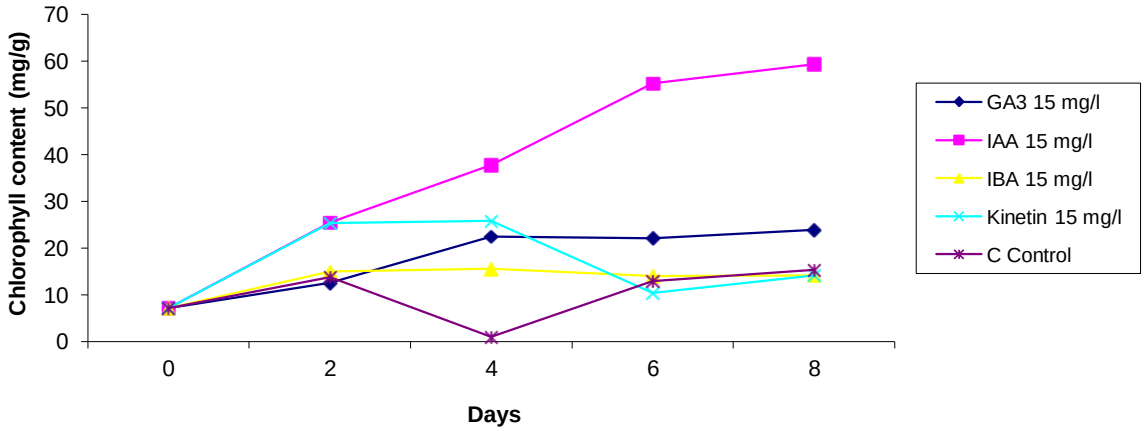

Figure 5. Effect of phytohormones on *Chlorella sorokiniana* IAM C212.

of the cells can be ranked as GA_3 > (Kinetin = IAA) >IBA. The best performing phytohormone was GA_3 at 20 mg/l concentration. This is in line with the report of Gonai et al. (2004). The harvesting of unicellular microalgae biomass is a challenging phase of the algae biomass production process (Wang et al., 2008; Brennan et al., 2012) and accounts for 20 to 30% of the total costs of production according to Gudin and Therpenier (1986). Small sizes of some algae cells make the recovery of biomass difficult (Chen et al., 2011). The discovery of phytohormones and the optimum concentrations that lead to increases in cell size of *C. sorokiniana* is very significant as it will lead to reduced cost in downstream processing of the microalgae biomass. Unfortunately, there was a negative correlation between the cell size of *C. sorokiniana* IAM C212 and biomass productivity in this study. GA_3 was the least effective in increasing biomass productivity but the most effective in increasing the cell size.

Effect of phytohormones on chlorophyll contents of *C. sorokiniana* IAM C212

The effects of the phytohormones on chlorophyll contents of *C. sorokiniana* followed the same trend as the dry weight with IAA at a concentration of 15 mg/l giving the highest value of 50.94 mg/g-cell biomass (Figures 5 and 6). This compares with the control (without

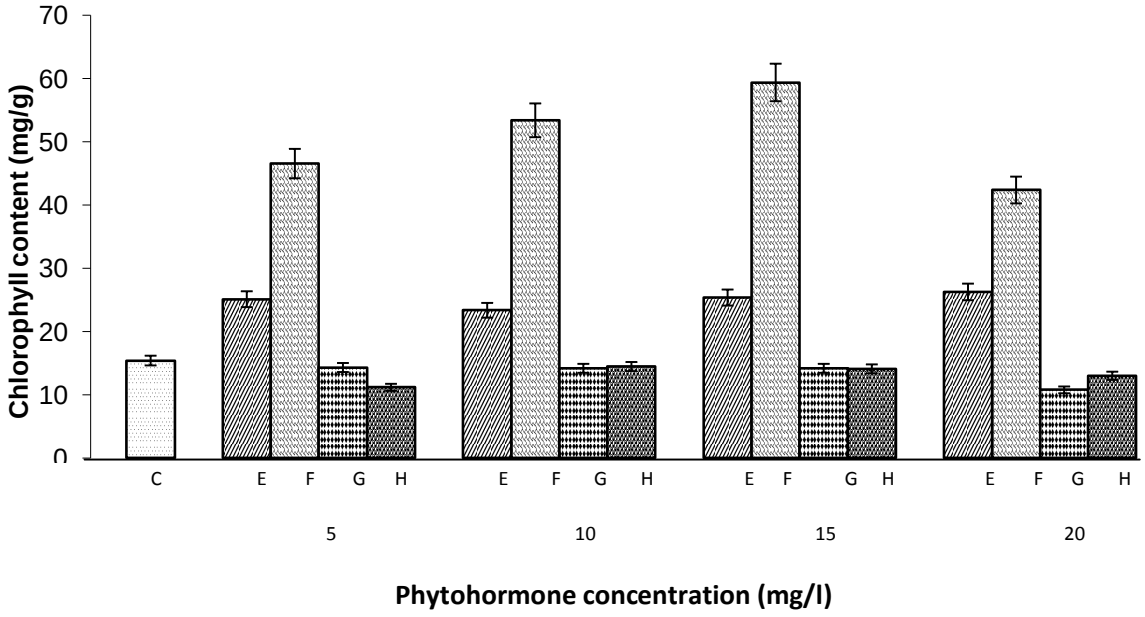

Figure 6. Effect of phytohormones on chlorophyll content of *Chlorella sorokiniana* IAM C212. Symbols: C, Control; E, GA_3; F, IAA; G, IBA; H, kinetin.

phytohormone) which had an average value of 15.40 mg/g. This represents more than 3-fold increase in chlorophyll contents over the control. However, it is only IAA and GA_3 that resulted in significant increase in chlorophyll contents of the cells. The effectiveness of the phytohormones in increasing the chlorophyll contents of the microalgae can be ranked as IAA (50.94 mg/g) > GA_3 (25.37 mg/g) > {Control (15.40 mg/g), IBA (14.30 mg/g), Kinetin (14.10 mg/mg)}. The effectiveness of the various concentrations of these phytohormones in increasing the chlorophyll contents of *Chlorella* can be ranked as 15 mg/l > (10 = 20 mg/l) > 5 mg/l. Surprisingly, GA_3 which was the least effective in enhancing biomass productivity was more effective than IBA in increasing the chlorophyll content. Presently, chlorophyll content of algae biomass has significant effect on their market values. Chlorophyll is an essential compound in many everyday products. It is used not only as an additive in pharmaceutical and cosmetic products but also as a natural food colouring agent. Chronic ulcer is a significant health problem in the society, with lengthy periods required for its treatment (Hosikian et al., 2010). Chlorophyll's ability to increase the rate of healing is a breakthrough for ulcer sufferers. Because of this property, chlorophyll is used not only in the treatment of ulcers and oral sepsis but also in proctology. Fruit and vegetable consumption has been associated with decreasing the risks of cancer. Phytochemicals present in these foods, particularly chlorophyll and its derivatives, have been suggested to play a key role in cancer prevention due to their antioxidant and antimutagenic activities (Lanfer-Marquez et al., 2005; Ferruzzi and Blakeslee, 2007). The discovery of a phytohormone that simultaneously leads to higher biomass production and high chlorophyll content is a useful contribution to advancing biotechnological applications of microalgae in cosmetic, pharmaceutical and food industries.

Effect of phytohormones on protein content of *C. sorokiniana*

The effect of phytohormones on protein content of *C. sorokiniana* is shown in Figure 7. The effects of the phytohormones on protein contents of the cells were not significant ($P > 0.05$). Treatment of *Chlorella* with 10 or 20 mg/l of GA_3, 15 mg/l of kinetin, and 15 mg/l of IBA or IAA gave protein contents of 46.64, 45.83 and 45.81%, respectively. These compare with the control which had 43.38% protein after eight days of cultivation. Although not statistically significant, GA_3 enhanced the protein content of *C. sorokiniana* despite its ineffectiveness in stimulating growth and biomass productivity. The results of this study imply that there is a positive correlation between the cell size of the green alga and their protein content. Salama et al. (2014) reported that the protein content of the microalga *Scenedesmus obliquus* was decreased at high concentration of IAA (10^{-5} to 10^{-6} M). This is in line with our finding in this research using *C sorokiniana* IAM C212. There is a negative correlation between cell growth rate and protein content as the fast growing cells tends to accumulate carbohydrates more

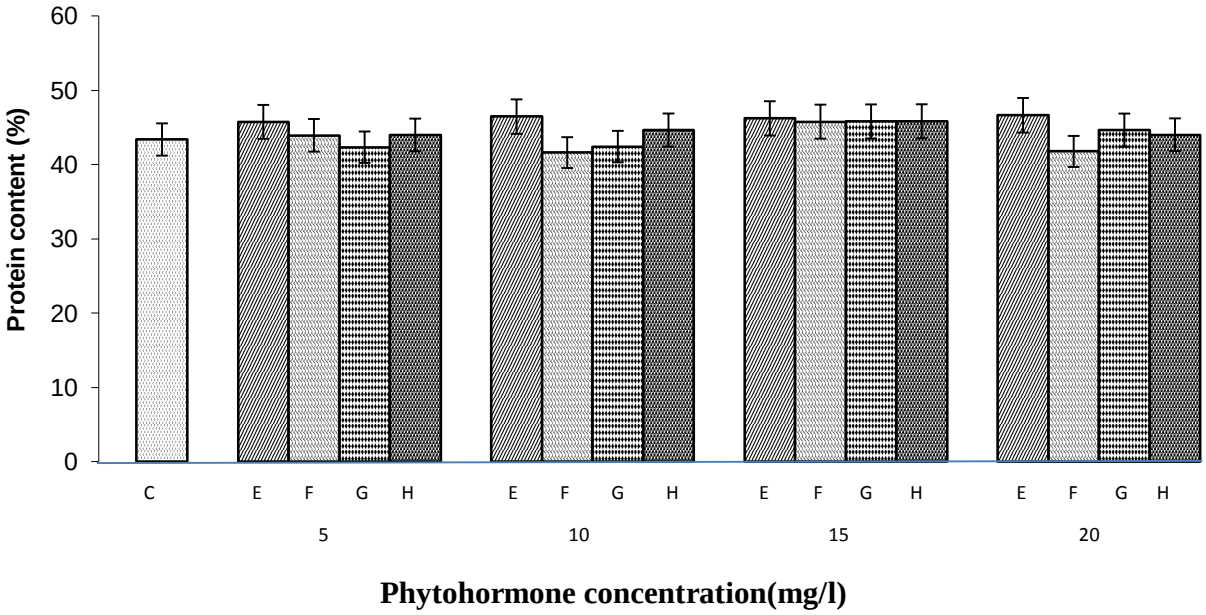

Figure 7. Effect of phytohormones on protein content of *Chlorella sorokiniana* IAM C212. Symbols: C, Control; E, GA_3; F, IAA; G, IBA; H, kinetin.

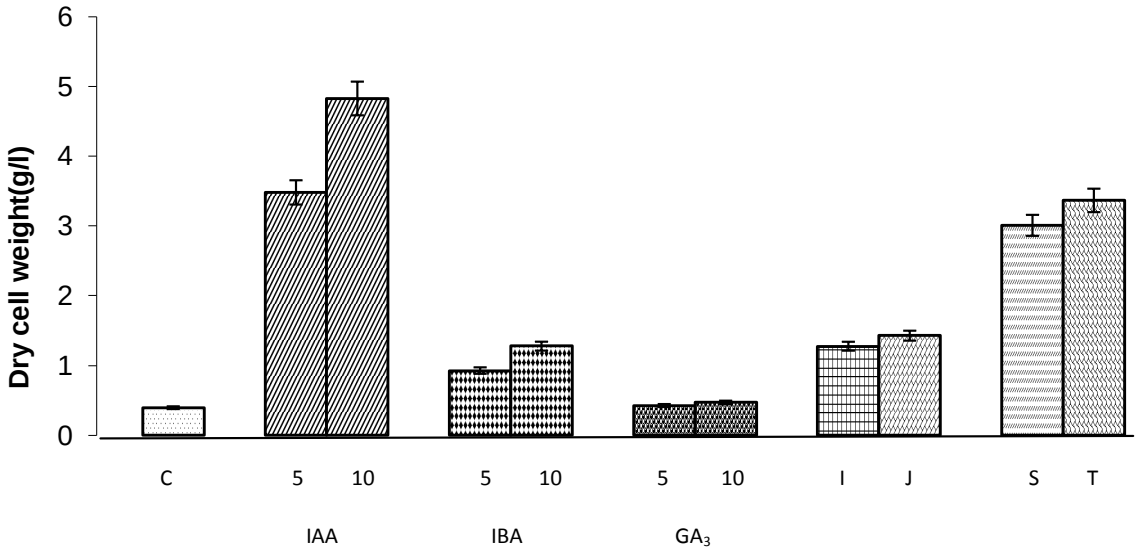

Figure 8. Effect of combined phytohormone on dry cell weight of *Chlorella sorokiniana* IAM C212. Symbols: I, IAA (10 mg/l) + GA (5 mg/l); J, IAA (12.5 mg/l) + GA (2.5 mg/l); S, IBA (10 mg/l) + GA (5 mg/l); T, IBA (12.5 mg/l) + GA (2.5 mg/l).

and slow growing cells tend to accumulate protein more (Markou and Nerantzis, 2013).

Effect of combinations of phytohormones on the dry cell weight of *C. sorokiniana*

The effect of combinations of phytohormones on the dry cell weight of *C. sorokiniana* is shown in Figure 8. The combination of phytohormones IBA (12.5 mg/l) + GA (2.5 mg/l) exhibited the highest effect with an average dry cell weight of 3.36 g/l after 8 days of cultivation. This compares with the control (without phytohormones) which recorded 0.394 g/l dry cell weight after 8 days of cultivation. Individually, neither IBA (1.66 g/l) nor GA_3 (0.47 g/l) at concentration of 15 mg/l did better than their

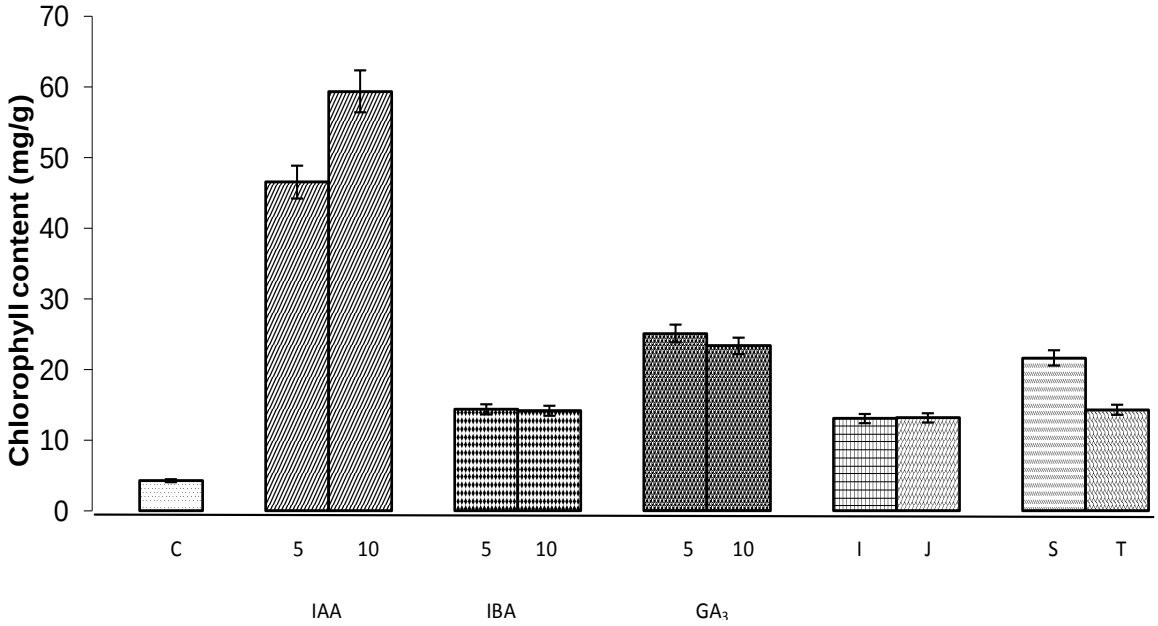

Figure 9. Effect of combined phytohormones on chlorophyll contents of *Chlorella sorokiniana* IAM C212. Symbols: I, IAA (10 mg/l) + GA (5 mg/l); J, IAA (12.5 mg/l) + GA (2.5 mg/l); S, IBA (10 mg/l) + GA (5 mg/l); T, IBA (12.5 mg/l) + GA (2.5 mg/l).

combination in enhancing cell concentration. There is a clear synergistic effect of combining IBA with GA_3 on cell dry weight as the resulting cell concentration (3.36 g/l) is 1.57 times higher than the sum of the cell concentrations obtained separately with the two phytohormones (1.66 + 0.47 = 2.13 g/l). IAA in all combinations with GA_3 did not exhibit any synergistic effect since the cell concentration obtained with IAA alone was higher than the values obtained with a combination of IAA (12.5 mg/l) and GA_3 (2.5 mg/l) or a combination of IAA (10 mg/l) and GA_3 (5 mg/l). As shown in Figure 9, the combination of IBA (12.5 mg/l) and GA_3 (2.5 mg/l); and IBA (10 mg/l) and GA_3 (5 mg/l), exhibited synergistic effect on *C. sorokiniana* in terms of biomass production and chlorophyll contents. Hunt et al. (2010) combined NAA (5 mg/l) and GA_3 (10 mg/l) and recorded about 38% increase in biomass productivity. To the best of our knowledge, no combination of GA_3 with IBA, a less performing member of auxin family, has been reported before now. In this study, we combined IBA and GA_3 and recorded over 7-fold increase in biomass concentration compared with GA_3 alone, and 1.96 fold increases over IBA alone. Furthermore, the combination of IBA and GA_3 simultaneously led to higher biomass and chlorophyll content, which is attractive for cosmetic and pharmaceutical industries. Hunt et al. (2010) reported that NAA in all combinations with GA_3, IBA, and zeatin showed only marginal increase in average productivity over the control.

Conclusion

Phytohormones can be used to increase the growth (dry cell concentration and cell number), cell size, protein and chlorophyll contents of *C. sorokiniana* IAM C212. However, the choice of the phytohormone and the concentration depend on the specific objective. Auxins (IAA and IBA) had pronounced effects on both dry cell concentration and cell numbers but only IAA had significant effect on the cell size. On the other hand, chlorophyll contents were more affected by IAA and GA_3. IAA and GA_3 have been reported to enhance cell elongation in higher plants and in this study, they also resulted in significant increase in the size of *C. sorokiniana*. Although kinetin is known to enhance cell division in higher plants, there was no significant effect of addition of kinetin to the medium on cell number of *C. sorokiniana.* Our results also show that there were synergistic effects of IBA and GA_3 on cell growth and chlorophyll contents of the cells.

Conflict of interests

The authors did not declare any conflict of interest.

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