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# Optimization of Sucrose Concentration and Light Treatment in Cell Suspension Culture Establishment of *Barringtonia racemosa* L.

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## Abstract

**Objective:** This study was carried out to optimize the concentration of sucrose and light treatment for the establishment of cell suspension cultures with the greatest records of dry cell biomass. **Methods:** The homogeneous cell suspension cultures of *B. racemosa* cultured in MS (Murashige & Skoog's) media with 1.0 mg/l 2,4-D (2,4-dichlorophenoxyacetic acid) and 1.5 mg/l kinetin were optimized for sucrose concentrations (2.0, 3.0 & 4.0% w/v) and light treatment (24-hour light or 24-hour dark). The dry cell biomass and pH values were recorded for 30 days. The data gathered were plotted against time and statistically analysed by analysis of variance (ANOVA). **Results:** The supplementation of 4.0% sucrose in 24-hour dark treatment recorded the most optimum dry cell biomass record. The different phases of cell growth could be identified from the growth curve constructed based on dry cell biomass records. The first two-day duration was identified as lag phase in all treatments tested followed by growth exponential phase and constant growth in cells until they achieved their maximum dry cell biomass. Afterwards, some of the treatments underwent rapid declining phase while others showed a short duration of stationary phase by depicting a plateau stage in the cell kinetic plot before finally entering the declining phase afterwards. It has been observed that the cells treated in the dark achieved their highest record of dry cell biomass earlier than those treated in the presence of light in the cultures supplemented with 3.0% and 4.0% sucrose. The effects of variations in sucrose concentration and light treatment were generally significant ($p < 0.05$) on dry cell biomass but were insignificant generally towards pH values ($p > 0.05$). **Novelty/Improvement:** A protocol for the establishment of cell suspension cultures of *B. racemosa* and the importance of sucrose and light treatments towards cell growth had been developed and highlighted.

**Keywords:** *Barringtonia racemosa* L, Cell Suspension Culture, Light Treatment, Sucrose Concentration

## 1. Introduction

Plant cell suspension culture is a type of applications in plant biotechnology which has gained attention and increasing interest since the last few decades. It has become a preferred platform over its traditionally practised natural harvest counterpart for the production of various valuable plant-derived products[1]. Cell suspension culture systems are used nowadays for large scale culturing of plant cells from which plant bioactive compounds could be extracted. A suspension culture is preceded by the induction of large amount of friable callus and afterwards, it is developed by transferring the relatively friable portion of the callus into liquid medium and is maintained under suitable conditions of aeration, agitation, light, temperature and other physical parameters[2] and further result in the formation of fast growing cultures of cells[3]. Among the major advantages of this approach are the sustainability features of the system to yield defined standard phytochemical and being independent from the limitations caused by microbial infections, pest infestation, geographical, seasonal and ecological factors[1,4].

In plant cell culture, sucrose composition is one of the chemical factors that should be considered and frequently assessed in numerous studies[5-8]. It has been well

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acknowledged that the use of sucrose in the media is preferred over other forms of carbohydrates. Essentially functions as a source of carbon, sucrose is favored due to its ease of availability, cost effectiveness and stability to autoclaving which is not easily caramelized after being autoclaved[9]. Besides, the suitability of sucrose to be used in cell suspension cultures had been proven when it did not cause growth cessation on prolonged sub culturing[10]. In terms of physical factor, the effects of light treatment may affect the growth of cells as well and the impact of light regime on plant tissue culture systems has been well documented in a number of previous researches[8,11,12].

The species used in the current study which is scientifically named as *Barringtonia racemosa* is a type of mangrove plant species. It has been well associated in traditional practices in various tribes around the globe and has been proven to be pharmacologically effective[13]. To date, the studies pertaining to *in vitro* culture establishment in *B. racemosa* are limited hence requires further development and optimization studies related to the area. The cell suspension cultures involved in this study were established from endosperm-derived friable calli grown on optimum treatment combination of plant growth regulators[14]. The relatively high concentration of phenolic compounds present in the species is anticipated to confer greater benefits for the secondary metabolites production in the culture system[15]. This current research was focusing on the screening and identification for the most optimum sucrose and light treatments in order to establish the viable cell suspension cultures of *B. racemosa* with the greatest cell biomass to ensure sufficiently produced cells in the cultures. It was carried out to obtain the optimum homogeneous cell suspension cultures and to identify the cell growth kinetic which is very much important for plant secondary metabolites studies to be further carried out in this species.

# 2. Materials and Methods

## 2.1 Initiation of Cell Suspension Culture

Friable calli with the weight of approximately 3.0 g were inoculated into 100 ml liquid culture medium treated with similar treatment used for callus induction[14] in the absence of Gelrite in 250 ml wide neck Erlenmeyer flask (Duran, Germany). The cultures were continuously shaken on shaking incubator (Daihan Scientific, Korea) at 100 rpm in dark at 25±2°C. After seven days, the cells were filtered through sterile stainless steel sieve to isolate the fine cells from the coarse, larger clumps of cell aggregates and an amount of 25 ml cell suspension culture was transferred into 250 ml wide-neck Erlenmeyer flasks which contained 25 ml fresh media. The cultures were further subcultured at every three-day interval by transferring 10 ml cell suspension cultures into 40 ml fresh liquid media. They were maintained under similar culture conditions. After completion of five passages of subculture whereby complete homogeneous suspension cultures were obtained, the cell suspension cultures were further used for the optimization studies.

## 2.2 Determination of Sucrose Concentrations and Light Treatments

The optimum hormonal treatment identified during optimization of hormonal composition phase (data not shown) was used with different combinations of sucrose concentrations and light factors. Three different concentrations of sucrose [2.0%, 3.0% and 4.0% (w/v)] which were constantly shaken on orbital shaker (PROTECH, Malaysia) under 24-hour light (950 lux) or 24-hour dark treatment (Daihan Scientific, Korea) were used to screen the most optimum treatment condition for rapid stationary phase attainment in cell suspension cultures establishment of *B. racemosa*. The treatments used were tabulated as presented in Table 1.

**Table 1.** Variation of treatments used for the optimization of sucrose and light treatment on cell suspension cultures of *B. racemosa*

| Sucrose concentration (% w/v) | Light treatment |
|---|---|
| 0 (Control) | 24-hour light |
| | 24-hour dark |
| 2 | 24-hour light |
| | 24-hour dark |
| 3 | 24-hour light |
| | 24-hour dark |
| 4 | 24-hour light |
| | 24-hour dark |

### 2.3 Identification of Cell Growth Phases and pH Values

Growth and development of cells in the suspension cultures were recorded by considering changes in the dry cell biomass of cell suspension cultures. The dry cell biomass were recorded at every two-day interval for a duration of 30 days and the data gathered were plotted to form a growth curve of dry cell biomass (g/l) over time (day). Distinctive different phases were determined from the cell growth curve by taking into account the shape of the curve produced which reflected the timely changes in the cell growth. The optimum treatment was identified by considering the greatest dry cell biomass recorded amongst all the treatments tested. In addition, the pH changes and cultures' opacity were also observed with the latter was done by comparing the colour intensity of the cultures over time.

### 2.4 Microscopic Observation of Cell Suspension Cultures

The cell suspension cultures were subjected to microscopic observation after 5, 20 and 25 days in culture. Evans blue dye was used as staining agent at a concentration of 0.05% (v/v). Accurately aspirated 1000 µl of cell suspension cultures at the respective growth phases were taken and mixed with three drops of Evans blue dye (0.05% v/v). The mixtures were incubated at room temperature (approximately 25°C to 27°C). After 15 minutes of incubation, the mixtures were viewed under Bright Field (BF) microscopy mode by using inverted microscope (Olympus, Japan). The cellular morphological differences between those phases were observed and noted.

### 2.5 Statistical Analysis of Results

Three replications were used for each treatment. The results were processed by Microsoft Excel for mean and standard deviation data. Then, they were analyzed by using IBM SPSS Statistics version 17.0 and ANOVA were applied. The data were analysed by using Two-Way ANOVA to figure out the effects of each factor (sucrose and light) on dry cell biomass and pH value. The mean difference considered as significant at 0.05 level ($p< 0.05$).

## 3. Results and Discussion

### 3.1 The dry cell biomass of cell suspension culture

Different treatments were found to produce almost similar onset of growth kinetic in each phase of cell growth with a very slight difference (2 days) from one treatment to another. Distinctive phases of cell growth in the cell suspension cultures could be identified from the growth curve constructed based on dry cell biomass records which were taken regularly for every other day (Figure 1). Generally, the first two days marked a distinctive phase of growth delay (lag phase) in all treatments followed by growth exponential phase and constant growth in cells until they achieved their maximum dry cell biomass. After the lag phase which was identified to occur during first two days in culture, cells were undergoing exponential phase on day two onwards until day 20 with the increment in the accumulation of dry cell biomass of up to 9.70 fold over the initial dry cell biomass. Afterwards, some of the treatments underwent rapid declining phase while others showed a short duration of stationary phase by depicting a plateau stage in the cell kinetic plot before finally entering the declining phase afterwards. It had been observed that the cells treated in the dark achieved their highest record of dry cell biomass earlier than those treated in the presence of light in the cultures supplemented with 3.0% and 4.0% sucrose.

All treatments showed a brief lag phase during the first two days in culture before starting exponential phase afterwards. Pertaining to sucrose composition, the concentration of 4.0% (w/v) sucrose was identified as the most optimum composition with the highest dry cell biomass record was achieved in the cell suspension cultures of *B. racemosa* both in the presence of light (14.99 ± 0.11) and dark (15.62 ± 0.31) at 22 and 20 days respectively. The present findings were almost consistent to that reported previously[7] which verified the concentration of 4.0% sucrose as the optimum composition suitable for biomass accumulation in *Withania somnifera*. Meanwhile, in a previous study, it had been found that the maximum cell growth was recorded on day 20 of culture in the cell suspension cultures of *Stevia rebaudiana* supplemented with 4.5% sucrose concentration[16]. In another study, it had been verified that sucrose supplemented at 4.24% (w/v) in cell suspension culture was the optimum concentra-

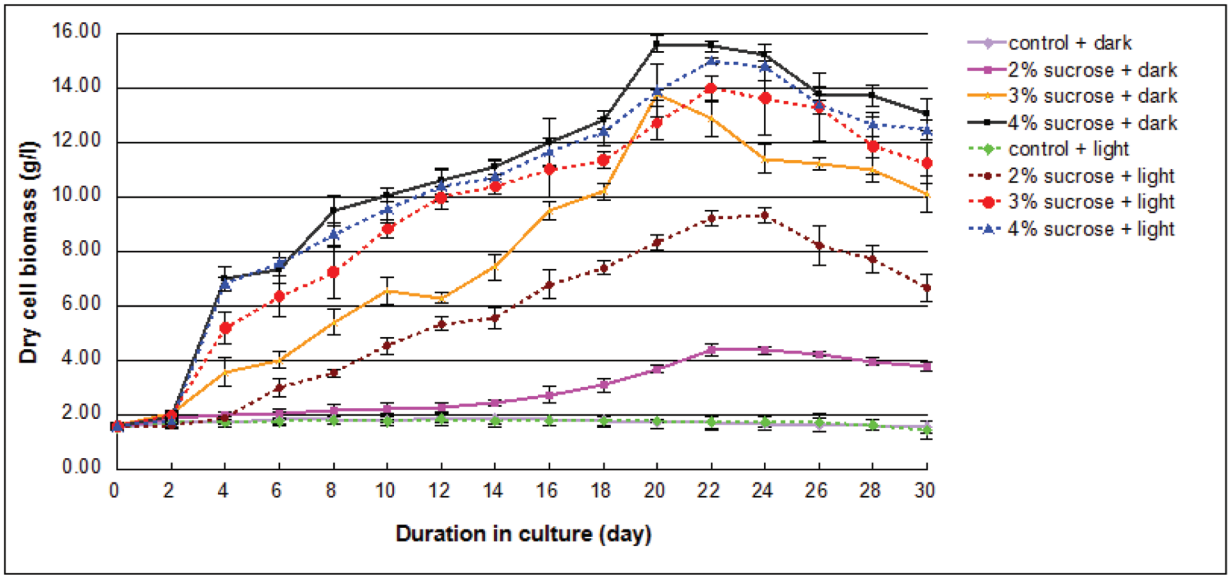


**Figure 1.** Dry cell biomass (g/l) of cell suspension cultures of B. racemosa in the media treated with 1.0 mg/l 2,4-D and 1.5 mg/l kinetin supplemented with different concentration of sucrose (2%, 3% and 4%) under 24-hour dark and 24-hour light treatment continuously agitated at a rotational speed of 100 rpm and maintaned at 25 ± 2 °C. Error bar represents standard error mean.

tion to support high cell biomass production in *Eurycoma longifolia* Jack[17].

In a study recorded on *Taxes cuspidata*[18], the accumulation of biomass in the cultures was closely related to the concentration of available carbohydrate in the medium and found to be in agreement with current findings. It could be observed in this study that the dry cell biomass were increased following the increase in the concentration of sucrose and similarly such correlation was previously reported[19] as well in their study on the suspension cultures of *Lonicera macranthoids*. Meanwhile, the lowest dry cell biomass records were found in the media with the lowest sucrose concentration (2.0% w/v) regardless of light factors. According to[5], high biomass accumulation could be achieved by using high sucrose concentration and the increasing pattern of biomass accumulation was observed following an increase in sucrose concentration which were ranging from 1.0% to 6.0% (w/v). However, the accumulation of biomass would be inversely related to the sucrose composition if the sucrose concentration is increased more than 6.0%[5,20] in which the phenomenon could be attributed to higher osmotic pressure and reduced nutrient uptake[21].

The importance of sucrose in providing energy to the cells had been described and mentioned in a number of studies[20,22] whereby sucrose is indeed recognized as an important carbon source, providing energy and playing a vital role in the synthesis of cell constituents. The supplementation of sucrose is critically essential for primary metabolism of plant cells in the culture medium since they lack autotrophic ability hence require external supply of carbon for energy as well as to support cell proliferation[23]. It had been stated that sucrose uptake was relatively fast in which all of sucrose had been absorbed or hydrolysed after approximately 6 days in the cell culture of *Taxus cuspidata*[18]. The sucrose supplied into culture medium is rapidly converted into glucose and fructose by hydrolyzing enzymes known as sucrose synthase and invertase[24] in which the utilization of fructose is normally preceded by glucose utilization[23,25].

With respect to the light factor, the treatments supplemented with 2.0% and 3.0% (w/v) sucrose and maintained in 24-hour light were found to record higher dry cell biomass than those cultured in the dark of similar sucrose concentration. In this study, white fluorescent lamp (950 lux) was used for the cultures maintained in 24-hour light treatment. These findings demonstrated that the presence of light is more favourable in producing higher biomass in the presence of sucrose at lower than 4.0% (w/v) concentrations. However, at 4.0% (w/v) sucrose concentration, greater maximum dry cell biomass were recorded (15.62 ± 0.31)in the cultures maintained in 24-hour complete darkness than those treated in 24-hour light treatment (14.99 ± 0.11). From all the treatments tested, the most

optimum condition which produced greatest maximum dry cell biomass was the cultures supplemented with 4.0% (w/v) sucrose maintained in the dark. Similarly, previous study on the cell suspension cultures of *B. racemosa* done by[26] also revealed that higher cell biomass was recorded in the cell suspension cultures maintained in the dark than in the light. Cell biomass grew almost six-fold higher when the cells cultivated in the dark as compared to those cultured in the light in the cell suspension cultures of *Arnebia* sp.[27]

In terms of the time taken to achieve maximum dry cell biomass, cultures supplemented with higher concentration of sucrose at 3.0% and 4.0% (w/v) maintained in the dark had shown earlier attainment of maximum growth as early as 20 days in culture than their maintained-in-the-light counterparts whereby the maximum dry cell biomass were attained on day 22 in culture. As for the media supplemented with 2.0% (w/v) sucrose, the cultures maintained in the dark achieved their maximum dry cell biomass records earlier (on 22 days) in culture than those cultured in the light (on 24 days in culture) (**Figure 1**). From such findings, it can be deduced that in the absence of light and at higher than 2.0% (w/v) sucrose concentrations, cells are being supplied with sufficient energy to facilitate rapid growth hence attaining their maximum dry cell biomass earlier (20 days in culture).

The influence of light on cell suspension cultures could not be generalized for all culture system since different species require different conditions to support optimum cell growth in suspension cultures. While some species prefer light for optimum cell biomass production, on the other hand, some show preference towards complete darkness. For the culture system that prefer light source in producing optimal biomass yield, it had been documented[28] that the reduction by 50% in the lighting area could cause a reduction of 13.3% biomass. On the other hand, the absence of light is required in certain species due to some growth promoting effects associated with darkness[29]. For instance, culture in dark may suppress unwanted differentiation process that may hamper the attainment of optimum cell suspension cultures and could prevent light-triggering effects on plant biological processes which may affect cell development[30] such as light-regulated cell death[31].

According to the statistical analysis of Two-Way ANOVA, the effects of variations in sucrose concentration and light treatment on dry cell biomass were generally significant ($p < 0.05$) during most of the times in the culture.There were also significant interactions identified between the effects of sucrose concentration and light treatment on dry cell biomass records. Based on Test of Between-Subjects Effects, wherein the effects of sucrose and light treatments were assessed according to the time in culture individually, there were no significant differences in the mean of dry cell biomass recorded during day 0 in culture. In addition, light also did not give significant difference ($p > 0.05$) on mean dry cell biomass during day two in culture as well. Nevertheless, sucrose and light exerted significant effects on mean dry cell biomass records in the rest of the time in culture. In a larger view of general perspective, it thus could be understood that varying sucrose concentration and light treatment could significantly influence the yield of dry cell biomass accumulation produced in the cell suspension cultures of *B. racemosa*.

## 3.2 The microscopic view and growth of cell suspension culture

In terms of microscopic views of cellular shapes, the cells during early phase of cell growth (early exponential) whereby the samples were taken during 5 days in culture were found to be mostly spherical and round in shape (**Figure 2A**). Subsequently, during early stationary phase wherein the cells were viewed after 20 days in culture, a mixture of cellular shapes was observed in which the presence of spherical and elongated cells were found to be equally existed and the aggregation of both were prominent (**Figure 2B**). Finally, at a later stage of growth, during late stationary phase (after 25 days in culture), the majority of cells were found to be elongated (**Figure 2C**). It had been previously mentioned that cells undergoing morphological changes in culture[32]. During exponential phase most of cells are small and rounded. And as a consequence of active cell division, eventually the cultures mostly composed of larger and elongated cells during stationary phase. In a documented review on culture characteristic[33], it had been said that plant cell elongation will occur after cell division ceases. Interestingly, it had been manifested that the present findings were consistent to what had been claimed in such review in which elongated cells were mostly identified during stationary phase; the point at which cell division are thought to be inactive or already ceased.

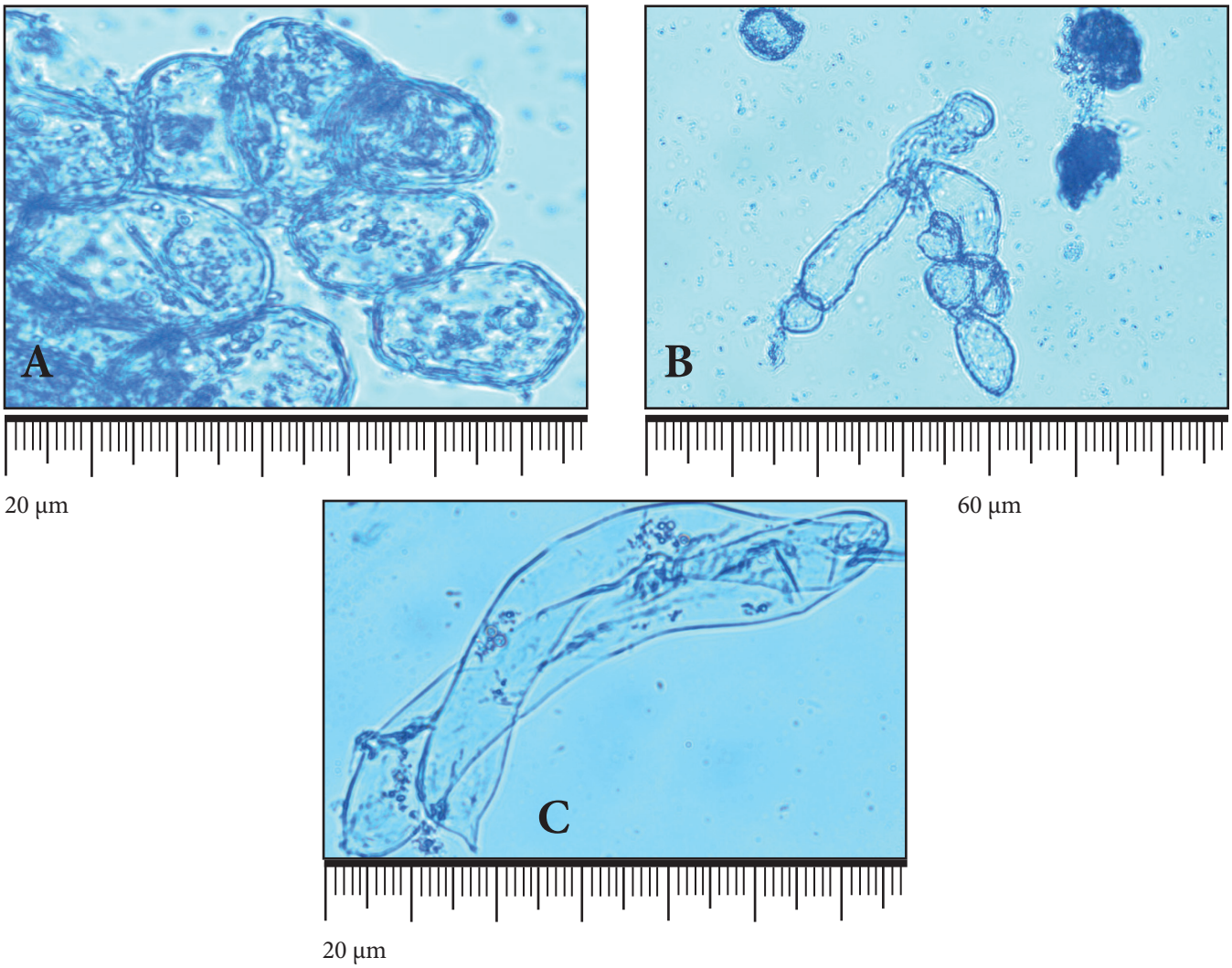


**Figure 2.** The appearance of cellular shapes of cell suspension cultures stained with 0.05% Evans blue dye under Bright Field (BF) microscopy in the most optimum treatment (1.0 mg/l 2,4-D and 1.5 mg/l kinetin in the presence of 4% sucrose maintained in 24-hour dark) during
[A] early exponential phase (5 days in culture) at 400x magnification,
[B] early stationary phase (20 days in culture) at 200x magnification, and
[C] late stationary phase (25 days in culture) at 400x magnification.

The different stages of cell growth could be noted by the differences in the colour intensity of the cell suspension cultures as well. The consistency of the cultures turned more viscous over times indicating cell growth has taken place (**Figure 3**). Finally, during declining phase, the cell suspension cultures were found to be obviously dark (**Figure 3D**). Apart from identifying the growth by the dry cell biomass records, the different stages of cell growth could also be noted by the differences in the colour intensity of the cell suspension cultures. The increase in cell biomass accumulation was apparently portrayed by the increase in culture opacity over the culture time in all treatments tested. Turbidity of suspension cultures serves as a visual determinant that could be used to demonstrate the growth of cells and to evaluate cell viability since suspension culture turbidity is directly proportional to the cell density[34].

The increase in culture opacity was observed in a timely manner in each treatment and it signified the increase in cell density as the cells grew progressively from one phase to another(**Figure 3A** to **3D**). Besides an increase in opacity, the cultures also turned darker from time to time and noticeably observed as they entered their declining phase of growth. The dark appearance of the

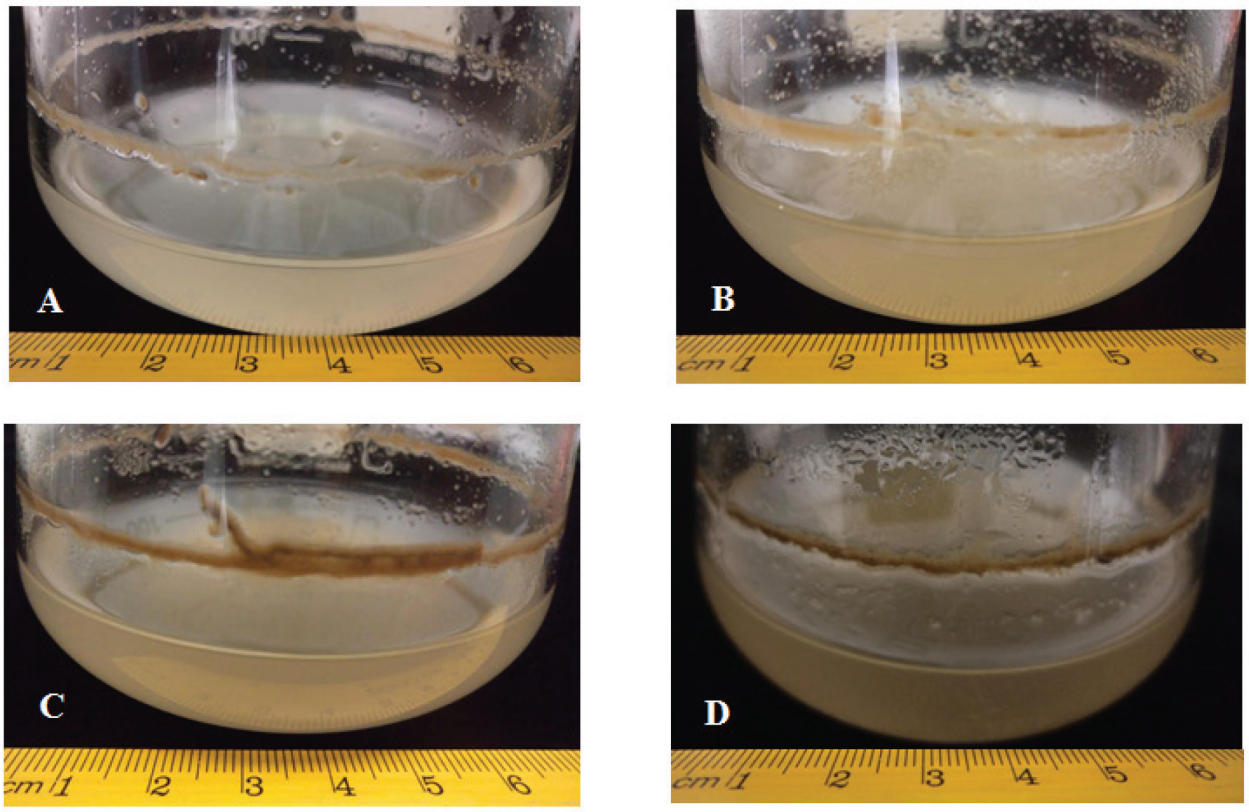


**Figure 3.** The appearance of cell suspension cultures in the most optimum treatment (1.0 mg/l 2,4-D + 1.5 mg/l kinetin in the presence of 4.0% sucrose maintained in the dark) during
(A) early exponential phase, (B) early stationary phase, (C) late stationary phase and
(D) decline phases whereby the photos were taken after 5, 20, 25 and 30 days in culture respectively.

cultures could be attributed to a higher density of cells in the suspension cultures with the accumulation of toxic metabolites and dead cells due to predominating depletion of nutrient in the medium[9]. In addition, the darkest appearance during declining phase could be related to the release of organelles from the dead cells into the media[35].

## 3.3 The changes of pH in the cell suspension culture

As for the pH records of cell suspension cultures, all treatments turned more acidic over time until the pH readings reached and remained approximately pH 3.2 in all of the treatments (**Figure 4**).It had been noted that there was a progressive reduction of pH over the time in culture identified in all treatments. In the beginning of media optimization study, the suspension cultures which were newly subcultured were having higher pH values ranging from 5.26±0.21 to 5.52±0.11 with only slightly lower pH than the newly prepared media (5.6 to 5.8). However, the pH turned more acidic and rapidly lowered after four days in culture and remained more acidic than initial pH until the end of cell growth phase in all treatments which were ranging from 3.14 ± 0.11 to 3.28 ± 0.27 at the end of observation period.

The declining of pH over time had been demonstrated previously in a number of studies for instance by[36] in which they found that after day 4 onwards, culture media were maintaining acidic pH (4.5-4.9) and declined gradually until the end of culture period in the cell suspension cultures of *Arnebia euchroma*. According to[28] the acidification of culture medium could be attributed to different cell uptakes of inorganic nitrogen source to ammonium ($NH_4$+) and nitrate ($NO_3$-). Medium acidification due to liberation of H+ into the medium could occur following preferential cell uptakes of ammonium over nitrate. Other than owing to inorganic nitrogen uptakes factor, according to[37] and[38], acidification and alkalinization of culture medium could also be attributed to nutrient absorption

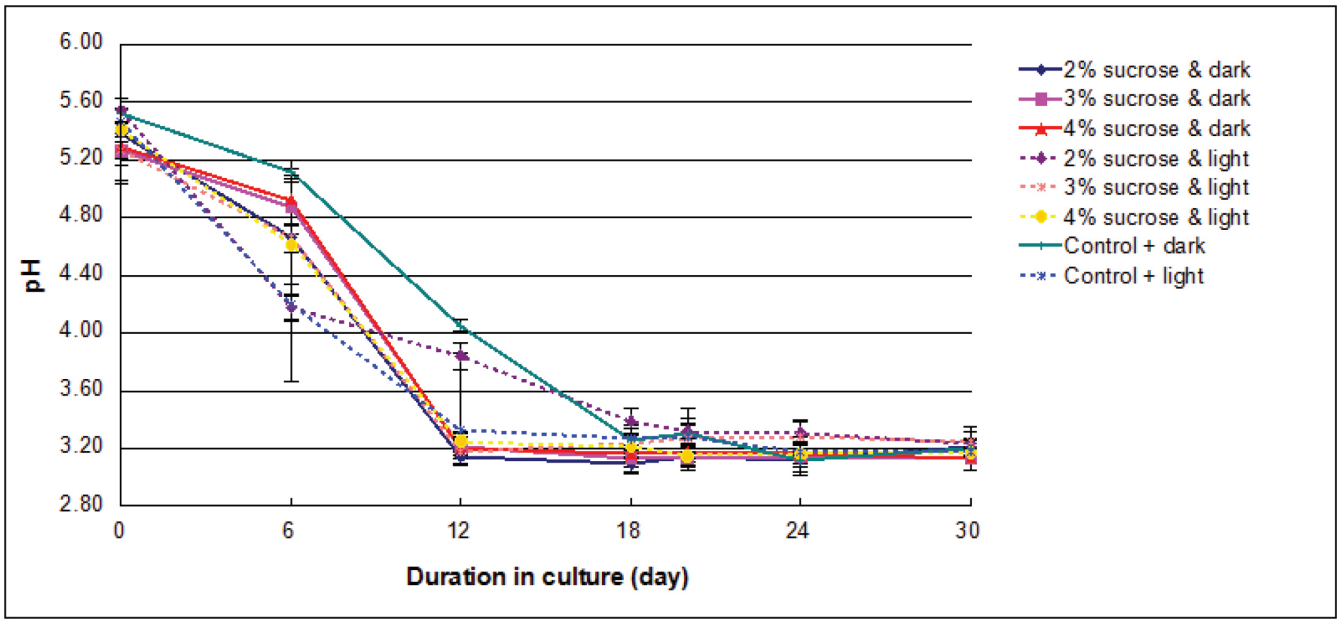


**Figure 4.** pH values of cell suspension cultures of *B. racemosa* in the media treated with 1.0 mg/l 2,4-D and 1.5 mg/l kinetin supplemented with different concentrations of sucrose [2%, 3% and 4% (w/v)] under 24-hour dark and 24-hour light treatment continuously agitated at a rotational speed of 100 rpm and maintained at 25±2°C. Error bar at each mean data point represents standard deviation.

as a function of ion exchange following deposition of free hydrogen ions ($H^+$) and hydroxyl ions ($OH^-$) as quoted by[39].

Besides, it could be inferred that the acidification was due to the secondary metabolite content of the cells which was possibly secreted into the culture medium and influenced the pH of the surrounding media. Even though secondary metabolites are normally secreted into the vacuole rather than medium, but the possibility of compound secretion into culture medium could also be taken into account[39-42]. For instance, it had been proven that anthraquinone of *Cinchona ledgeriana* cells were excreted into liquid medium[43]. For this present instance, it could be due to the secretion of gallic acid which is identified as one of main phenolic acids found in *B. racemosa* and it is therefore known to be acidic in nature.

While sucrose and light produced significant effects on cell biomass yield in plant cell suspension culture of *B. racemosa*, they are however did not influence pH values significantly in all treatments involved in the current study. According to the Two-Way ANOVA analysis, the pH values were not significantly differed ($p > 0.05$) following variations in sucrose concentrations and light treatments. Thus it could be deduced that the pH fluctuation patterns in the cultures of the present study were not significantly influenced by sucrose and light factors. Even so, the variation in pH reading is indeed species-dependent with different patterns of medium pH changes were identified in different plant species[40].

## 3.4 Other practical considerations in the cell suspension culture establishment

In this optimization studies, the initiation of culture system by using 3.0 g friable endosperm-derived calli was essential in order to have sufficient amount of cells. Initial cell density has been regarded as one of the most important factors in determining growth kinetic of cell suspension culture. It has been described by[23] that initial cell density of cell suspension culture is among the most pertinent elements in determining the duration of incubation period between culture initiation and stationary phase attainment. They mentioned that the prolonged lag phase and exponential phase of cell suspension cultures could be resulted from low initial cell densities and similar insight had been discussed by[44] as well in elaborating the process of plant cell culture establishment. A research done by[45] on *Artemisia annua* L. had shown that despite accumulating greatest growth index and cell biomass, the cultures with the least inoculums density were having prolonged lag phase and took longer to achieve

their maximum cell growth rate than those cultured with greater inoculum density.

Nevertheless, opting for the quantity of callus necessary to initiate the suspension culture should be accurately determined. While excessively dense cells would cause necrosis and dramatic decreasing of growth, too low density on the other hand would be insufficient for the cells to start division and proliferation process[46,47]. In discussing the matters,[48] had supported the use of a denser seeding of callus as compared to a lower initiating callus and suggested 2 to 3 g of friable callus per 100 ml of medium as the optimum amount for the establishment of healthy suspension culture. Therefore, by considering such factor, this optimization study were carried out by using 3.0 g calli during suspension culture initiation as an attempt to produce rapid stationary phase attainment.

With respect to subculture frequency, acording to[49], lengthy subculture interval may slow down cell division. Thus, in addition to using greater mass of initiating callus, it can also be inferred that the extended phases of growth could be made shortened by increasing the frequency of subculture of cell inoculum. Therefore, the cell inoculum from which the cultures for optimization studies were taken were subcultured at every-three-day interval. In the present findings, the attainment of stationary phase was successfully achieved as early as 20 to 24 days. Indeed, the importance of subculture frequency was clearly portrayed in the present findings and the rapid attainment of stationary phase is one of the important aspects which is beneficial for secondary metabolites study.

The sub culturing procedure is indeed important since it causes the growth of cells to be activated each time they are transferred into fresh liquid media[50]. According to[51], cells should be subcultured at weekly intervals or less if they are to be used for experimental purposes and in order to maintain the cultures in continuous exponential phase, they need to be subcultured frequently for every two to three days. Previously, a study done by[52] had demonstrated that the enhanced growth-promoting activity was revealed by using a culture with low density cells and could be achieved by frequent repetitive transfer. Furthermore, for the biochemical and physiological studies to be carried out in cell suspension cultures, they need to meet some important criteria in which they should have rapid cell growth, small cell size and require frequent subculture[53]. Nevertheless, care should be taken to avoid cells from being over diluted since the very low density cultures will result in the growth progress becomes slow[54].

## 4. Conclusion

From the current study, it is evident that the cell suspension cultures of *B. racemosa* cultured in the MS media treated with 1.0 mg/l 2,4-D and 1.5 mg/l kinetin and supplemented with 4.0% sucrose maintained in complete darkness and agitated at 100rpm at 25±2°C yielded the highest record of dry cell biomass. The findings related to cell suspension culture establishment in this study are imperative for subsequent works regarding bioactive compound production to be conducted in this tropical medicinal plant species.

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