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Effect of different LED lights spectrum on the *in vitro* germination of gac seed (*Momordica cochinchinensis*)

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Abstract

Momordica cochinchinensis, also known as gac, is an indigenous fruit that can commonly found in Southeast Asia. Studies had shown that *M. cochinchinensis* contained a higher level of carotenoids, β-carotene, and lycopene compared to other vegetables and fruits. The present study was conducted to study the effect of light conditions on gac seeds germination and effect of light-emitting diode (LED) spectra (violet, blue, green, and red) on shoot, root, and leaf formation from germinated gac seeds. A total of 60 surface sterilised uncoated gac seeds were cultured in MS media where half of them exposed to sunlight while another half kept in the enclosed cupboard. Germinated seeds were then transferred to new MS media which contained 1 mg/L of BAP and cultured under different LED lights. Gac seeds germinated well in 12 hours light treatment at the rate of 75% after one week while dark treated seeds did not germinate at all. The green LED light spectrum was the most effective for the production of the highest mean number of shoots at 4.75 ± 0.63. On the other hand, the violet LED was the most effective spectrum in producing the highest number of roots, which was 7.50 ± 0.58. Meanwhile green LED produced the highest root length of 6.25 ± 0.25 cm. Besides, green LED treatment also able to induce the highest number of leaves, which was 10.21 ± 1.89. Lateral shoot and tendrils were developed on blue LED spectrum treated seed. In conclusion, light facilitates gac seed germination while the green LED light induced better characteristics of gac plant.

Keywords: *Momordica cochinchinensis*, Seed, *In vitro* germination, LED.

Introduction

Momordica cochinchinensis is an indigenous fruit that can commonly found in most of the Southeast Asia countries such as Vietnam, Thailand, Cambodia, Laos and some parts of China (Chuyen *et al.*, 2015). *M. cochichinensis* Spreng. was discovered in Vietnam and it is commonly known as gac which was originated from the word "gấc" in Vietnamese (Kha *et al.*, 2011). Gac is a woody dioecious perennial plant which favours temperate areas and only produces fruits once a year which peaks in December and January where the average temperature is relatively lower than other months (Tran *et al.*, 2008).

Gac fruit has high demand in the market, including the West and Asia countries and can be consumed directly or used as an ingredient in cooking. Aside from its function as a natural red colourant, gac fruits also contain high contents of various types of carotenoids and other nutrients. The high level of carotenoids, β-carotene and lycopene in gac has been proven to improve human immune system and acts as a rich source of vitamin A (Vuong & King, 2003). Besides, gac fruits also possess antioxidant and anti-inflammatory properties that able to delay ageing and reduce pain, respectively (Mai *et al.*, 2016). In order to meet the market demand, growers from all around the world are trying alternatives to produce gac fruits with high quality. The present paper advocates the superiority of light-emitting diodes (LEDs) in enhancing the gac seeds germination and their subsequent development.

Gac can be propagated through the seeds but this method retain some uncertainties due to the dormancy property of the seeds. The seeds required 7 to 14 days or more in order to germinate. Alternatively, the plant can be cultivated from root tubers. High temperature between 60-70˚C is needed in order to physically break the dormancy of the seeds by removing the coarse and thick seed coat. Seed germination is dependent on environmental temperature, humidity as well as the storage period which should be optimised to ensure the germination. Rooted vine cuttings are more commonly used for propagation because this method is more reliable than production from seeds (Reynolds & Wardle, 2001). One of the benefits of grafting is that it is possible to graft female scion material onto the main shoot of the unwanted male plant, making it productive without destroying the male plant. Nevertheless, grafting retains several drawbacks as it exposes the cuttings to microbial pathogens and leads to pathogenic infections. Furthermore, the availability of gac plants that serve as the rootstocks is often regionally restricted (Tran et al., 2020). Hand pollination is useful during the flowering period as gac is a

dioecious plant where male and female flowers are in a different individual.

One of the factors that influences seed dormancy is the thick seed coat that is impermeable to water. It prevents the penetration of water into the seed, which is necessary to break dormancy. Gac seeds break their dormancy mainly under warm temperature, germination temperature may differ for individual plant species though. Gac can be grown as a non-flowered perennial plant in a cool area which takes more time for seed germination. Micropropagation is an alternative method to substitute traditional propagation ways which required longer time and larger labour (Honda *et al*., 2001). Among the common plant parts that used in plant tissue culture, such as seeds, shoot tips, nodal segments, leaves and roots, seeds are easier to handle and provide much more guarantee production compared to other plant parts.

Over the years, the effects of light-emitting diodes (LEDs) in agriculture have been studied and proposed as an alternative light source for *in vitro* plant growth and development (Bula *et al.*, 1991). LEDs have wavelength specificity, durability, small size, long operating lifetime, relatively cool emitting surface, and a photon output that is linear with the electrical input current, and the ability to control spectral composition (Brown *et al*., 1995) which made it became popular in the agriculture industry. LED-based sources are useful as it may control the growth and development of plant cell, tissue, and organ cultures by triggering physiological reactions (Kurilčik *et al.*, 2008).

The main aims of this study are to determine the effect of light conditions on *M. cochinchinensis* seed germination, and to induce multiple shoots, roots, and leaves formation from the seeds of *M. cochinchinensis* under different LED light spectra.

Results

Surface sterilisation of seeds

Total of 86.67 % of surface sterilised seeds was survived while 13.33 % were contaminated from the total of 60 seeds. Pink border was found to be surrounding the seeds, and reddish to pink marks were found on the damaged seed surface. Black or white furry materials were found on the edge of culture dishes.

Effect of light conditions on seed germination

After the first week, some seeds started to germinate. Uncoated *M. cochinchinensis* seeds germinated well under the illumination of light compared to dark treatment (Table 1). Seeds treated with 12 hours daylight germinated at a rate of 75 % after one week while dark treated seeds did not germinate at all. Dark treated seeds started germinating only after three weeks at the rate of 25 % while seeds treated with 12 hours daylight daily reached 100 % germination rate since the 2nd week.

Effect of LED light spectra on shoot multiplication

After three weeks of incubation on MS medium supplemented with BAP, seeds produced stem with powdery substances on the surface of cotyledons (Fig 1).

Seeds treated under green LED light produced higher number of shoots (4.75 ± 0.63). Meanwhile, no shoot was induced from the seeds under white light treatment (Table 2). Lateral shoots and tendrils were found to be formed on blue LED treated seeds (Fig 2). On the other hand, seeds treated with different LED spectra showed no significant difference for the length of stem.

Effect of LED light spectra on root elongation and multiplication

All seeds produced tap roots and adventitious, fibrous roots after four weeks of incubation (Fig 3). The seeds treated with a violet LED light produced a significantly higher number of roots (7.50 ± 0.58) as compared to the control treatment with only 4.50 ± 2.38 (Table 3). There is no significant difference between blue, green, and red LED light treatments in terms of root number.

On the other hand, seeds treated with green LED light displayed significantly higher root length, which was 10.21 ± 1.89 cm (Table 3). Meanwhile, violet and blue light treatments showed a lower root length which is 6.11 ± 1.54 and 5.99 ± 1.07 cm, respectively. There is no significant difference between red LED light and control treatment in term of root length.

Effect of LED light spectra on leaves multiplication

After three weeks of incubation on MS medium supplemented with 1 mg/L of BAP solution, most of the seeds produced leaves. Seeds treated with green LED light showed the highest number of leaves which is 6.25 ± 0.25 (Table 4). Meanwhile, control treatment showed the lowest mean number of leaves at 2.00 ± 0.00. The number of leaves produced under violet and blue LED light treatments was 4.50 ± 0.87 and 4.00 ± 1.58, respectively. There is no significant difference between violet and blue LED light treatments in terms of the numbers of leaves (Table 4).

Seeds cultured on MS medium supplemented with 1 mg/L of BAP solution showed physical differences among the seedlings under different treatments (Fig 4). Size of the leaf was too small for almost every culture which unable to collect data for analysis purpose.

Discussion

Surface sterilisation of seeds

Contaminated seeds with pink border and reddish pink marks were suspected to be caused by endophytic bacteria which appeared from the inside of the seeds. Black or white furry materials were suspected to be formed due to the growth of aerial fungus mycelia and spores.

Effect of light conditions on seed germination

The exposure of seeds under light condition enhances the germination rate, probably due to the activation of photoreceptor phytochrome B (PHYB) which mediates abscisic acid (ABA) and gibberellic acid (GA) signalling and metabolism. The upsurge of phytohormones promotes the growth and development of the embryo and improves the germination capacity. As indicated by Cho and colleagues (2012), the light-dependent activation of PHYB tends to increase GA levels through the removal of repressive histone arginine methylations which in turn promotes seed germination. In nature, light acts as one of the signalling factors for seeds embedded in soils. The irradiance of light for seeds located near to soil surface might be perceived as an environmental cue, wherein the conditions are optimal to

break seed dormancy as well as their further germination. This is especially important for small-seeded species, as small seeds often have limited resources, and these seedlings could not emerge successfully if they are embedded too deep into the soil (Fenner & Thompson, 2005). In plants, photoreceptors such as phytochrome are particularly vital in seed germination (Shinomura, 1997). Under far-red illumination or dark condition, the active form of phytochrome, Pfr converts into its inactive form, Pr. Nevertheless, the conversion among Pfr and Pr is reversible. The illumination of white light provides the red spectrum necessary to convert Pr into the active form, thus Pfr prevails over Pr under light condition. The accumulation of phytochromes in their active form will subsequently stimulate various cellular responses such as seed germination and flowering.

Effect of LED light spectra on shoot multiplication

Three weeks incubation was not enough for secondary shoots to completely generate. However, axillary buds which potentially grow and form shoots were induced (Beveridge, 2006). Seeds treated under green LED light produced the highest amount of axillary buds compared to other treatments. Green light is often considered as a spectrum with no significant role in supporting plant growth and development. However, green light supplementation could travel further than other spectra within the visible light and provides the plants with photons necessary to excite the electrons for photosynthesis to take place. In plants, the visible light wavelength which coincides with photosynthesis action spectrum is actively absorbed by photosynthetic pigments such as chlorophyll and carotenoids. This restricts the light absorption for plant parts located further away from the illumination source. Thereby, the residual green light could be utilised to support photosynthesis for these plant tissues and enhances the overall photosynthesis rate (Terashima et al., 2009). In the present study, the engagement of green LED might have promoted the photosynthesis of the *in-vitro* cultures as well as the production of photoassimilates which promotes the development of the axillary buds. Furthermore, the formation of tendrils and lateral shoot can be observed only under blue LED light treatment indicating explants grow faster under this illumination compared to other treatments.

Effect of LED light spectra on root elongation and multiplication

Root growth is primarily promoted by photosynthetic activity, and phytochrome A stimulates root growth independently of photosynthesis (Kurata & Yamamoto, 1997). Seeds treated under violet LED light produced the highest number of roots while seeds treated under green LED light produced the longest root length. In the present study, violet LED significantly promotes root formation. This can be attributed to the spectrum radiated by this particular light treatment. Both red and blue regions of the visible light spectrum provide the optimum absorption spectrum for the uptake of photosynthetic pigments. Thereby, violet irradiance elevates the photo-excitation in the photosystems and promotes photosynthesis rate. This further increases the availability of carbon and energy source needed to support plant growth and development, as demonstrated in the present study. The nutrients synthesised during photosynthesis tends to be allocated through phloem down to the lower plant parts such as roots and utilised for cellular functions as indicated by the source and sink mechanism. Blue LED light treatment displayed average growth of shoots. The findings of the present study is consistent with that of Ramírez-Mosqueda and co-workers (2017), which stated that blue LED light spectrum helps in stimulating shoot and root formation rate as well as shoot elongation during the rooting phase.

Effect of LED light spectra on leaves multiplication

Green LED light-treated seeds produced the highest number of leaves, which facilitates respiration and photosynthesis. However, as indicated by Zheng and Van Labeke (2018), blue LED light possesses a favourable effect on the anatomical development of the leaves. Along with far-red and red light, the green light is perceived by plants as a signalling factor which activates several shade avoidance mechanisms. In the present study, green light irradiances induce the formation of leaves, which could have been arisen as a result of shade avoidance action displayed by the cultures. Under conditions with limited access to an adequate illumination source, plants develop various physiological changes which include elongation of various plant parts such as hypocotyls, internodes and petioles. Plants exhibit tolerance to shade also by reducing branching and early flowering (Pierik and Wit, 2014). Large leaves formation under green light could be one of the physiological responses that emerge due to shade avoidance actions. In order to counteract with the light stress, the cultures allocate more of the resources for leaves formation. The increment of leaves number promotes the photosynthetic apparatus content and heightens the rate in capturing any available light and ultimately ensures the survival of the cultures. Nonetheless, the capacity of the green spectrum in enhancing photosynthesis rate (Terashima et al., 2009), could also be accredited for the higher leaves formation rate.

Materials and Methods

Plant materials and culture conditions

The seeds of *M. cochinchinensis* were collected from the ripened gac fruits of mother plants grown in Main Campus of Universiti Sains Malaysia, Penang, Malaysia. The healthy undamaged seeds of *M. cochinchinensis* were cultured on full strength Murashige & Skoog medium (1962). Total of 60 seeds, 30 for light treatment and another 30 for dark treatment, were prepared. The media were supplemented with 30 g/L sucrose and 2.75 g/L of the solidifying agent, Gelrite^{TM} (DUCHEFA, Netherlands). The pH of the media was adjusted to pH 5.7 - 5.8 using 1 M sodium hydroxide (NaOH) or 1M hydrochloric acid (HCl). The media were then autoclaved (Tomy ES-315) at 121 ^{0}C for 15 minutes.

Surface

Sterilisation of seeds

The seed coat (1.5 – 1.8 cm in diameter) was removed by using nutcracker. These uncoated seeds were brushed gently by using paintbrush in Sunshine® liquid dishwashing soap and 3 drops of Tween-20 to remove dust and dirt. The seeds were then washed under running tap water for 30 minutes. The seeds were swirled gently in 70 % (v/v) ethanol solution for 10 min, 20 % (v/v) Clorox solution for 10 min, and washed five times with sterile distilled water. Sterilised seeds were then incubated on full strength MS medium in

Table 1. Effect of different light conditions on the germination rate of gac seeds.

Treatments	Germination rate (%)		
	Weeks		
	One	Two	Three
Light	75	100	100
Dark	0	0	25

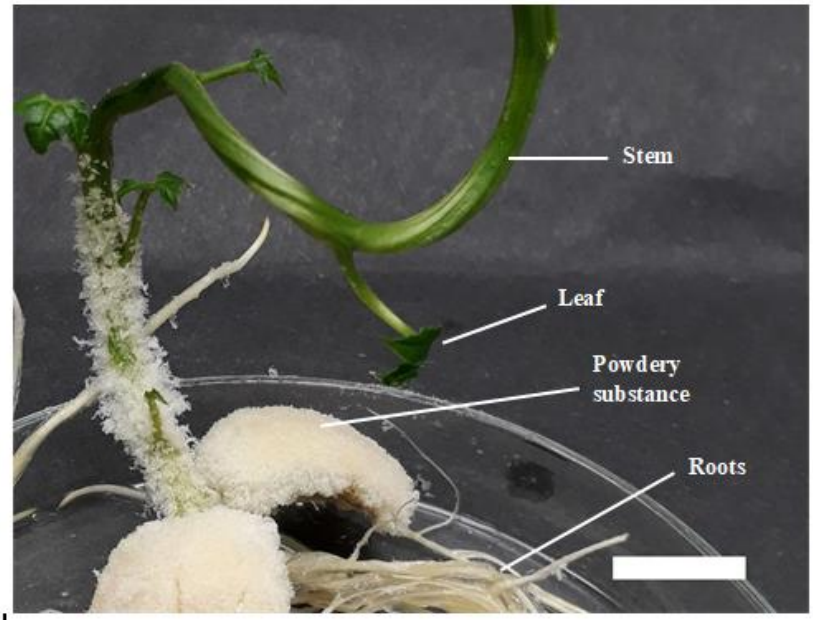

Fig 1. *In vitro* germination of *M. cochinchinensis* seeds after three weeks of culture. (Scale bar = 1 cm).

Table 2. Effect of different LED light spectra on the number of newly induced shoots of *M. cochinchinensis* after three weeks of culture.

Light spectra	Mean number of secondary shoots
Violet	1.00 ^{b} ± 0.41
Blue	2.25 ^{b} ± 1.31
Green	4.75 ^{a} ± 0.63
Red	1.50 ^{b} ± 0.87
White	0.00 ^{b} ± 0.00

*Means with different alphabets are significantly different. (Mean ± S.E.).

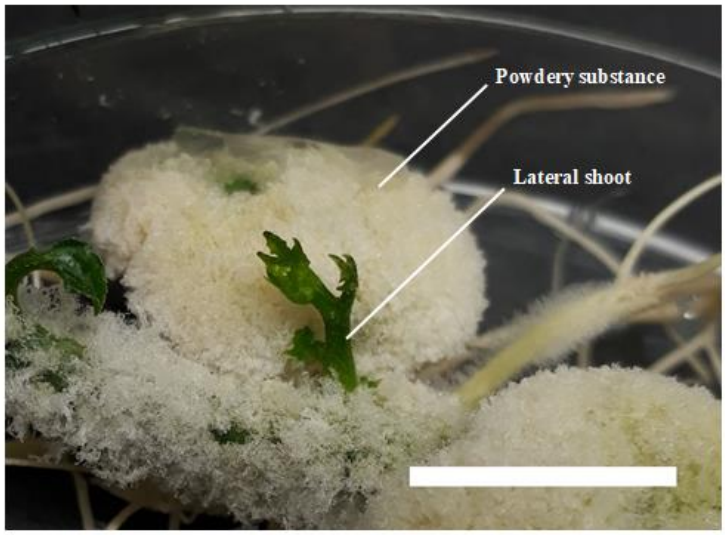

Fig 2. Formation of lateral shoot on blue LED light treated seed after three weeks incubation on MS medium supplemented with 1 mg/L of BAP. (Scale bar = 1 cm).

Table 3. Effect of different LED light spectra on the number of newly induced roots and average root length of *M. cochinchinensis* after three weeks of culture.

Light spectra	Number of roots	Mean average root length (cm)
Violet	7.50 [a] ± 0.58	6.11 [ab] ± 1.54
Blue	5.25 [ab] ± 1.89	5.99 [ab] ± 1.07
Green	5.50 [ab] ± 1.30	10.21 [a] ± 1.89
Red	6.50 [ab] ± 1.73	4.78 [b] ± 1.29
White	4.50 [b] ± 2.38	4.66 [b] ± 1.80

*Means with different alphabets are significantly different (Mean ± S.E.).

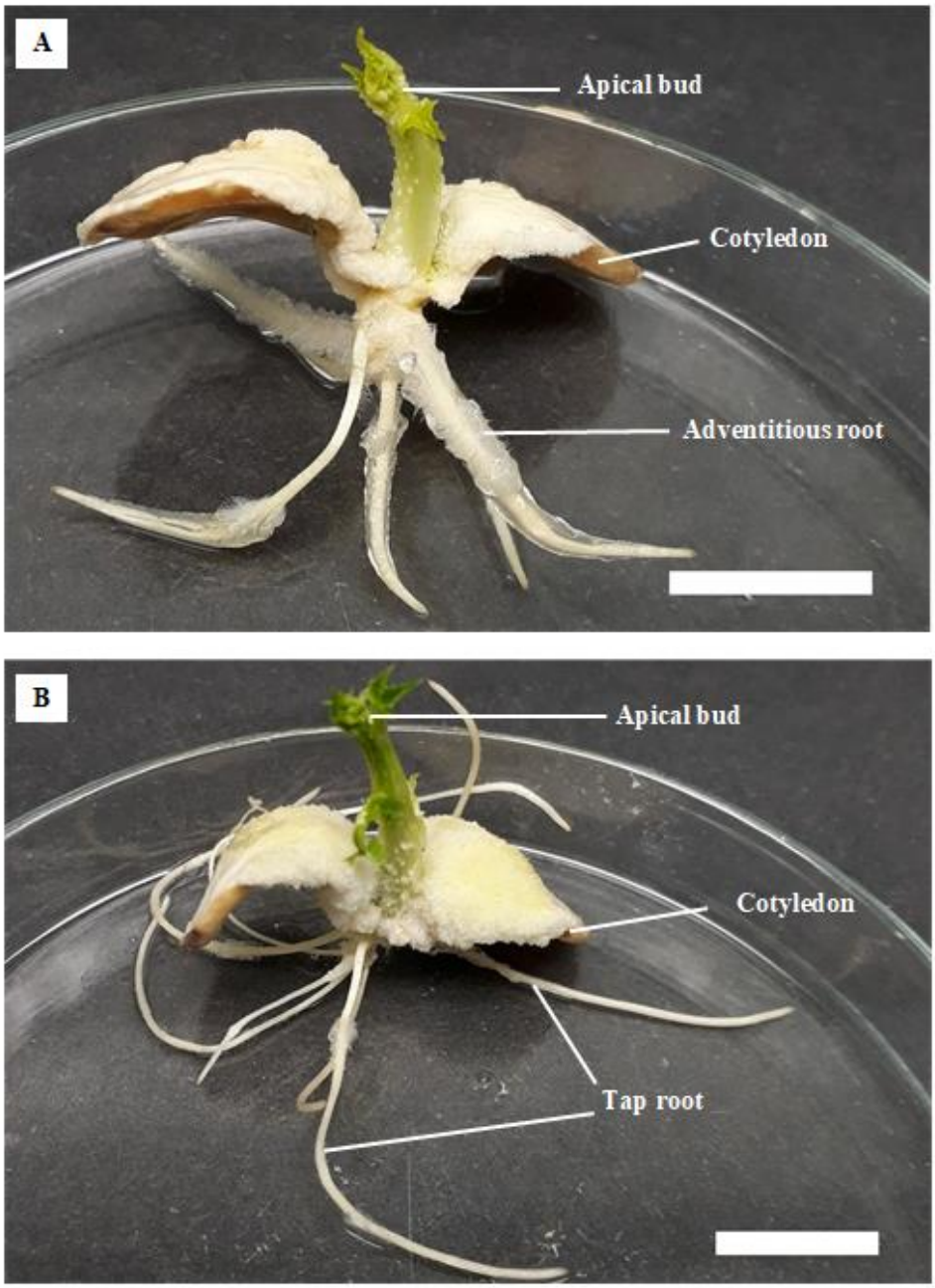

Fig 3. Effect of LED light spectra on root induction of *M. cochinchinensis* seeds. (A) *In vitro* apical bud with adventitious fibrous roots, and (B) *In vitro* apical bud with tap roots. (Scale bar = 1 cm).

Table 4. Effect of different LED light spectra on the number of newly induced leaves of *M. cochinchinensis* after three weeks of culture.

Light spectra	Number of leaves
Violet	4.50 ^{ab} ± 0.87
Blue	4.00 ^{ab} ± 1.58
Green	6.25 ^{a} ± 0.25
Red	2.50 ^{b} ± 1.44
White	2.00 ^{b} ± 0.00

*Means with different alphabets are significantly different. (Mean ± S.E.)

Table 5. Wavelength of selected LED light spectra.

Light spectra	Wavelength, nm
Violet	380 - 450
Blue	450 - 495
Green	495 - 570
Red	620 - 750

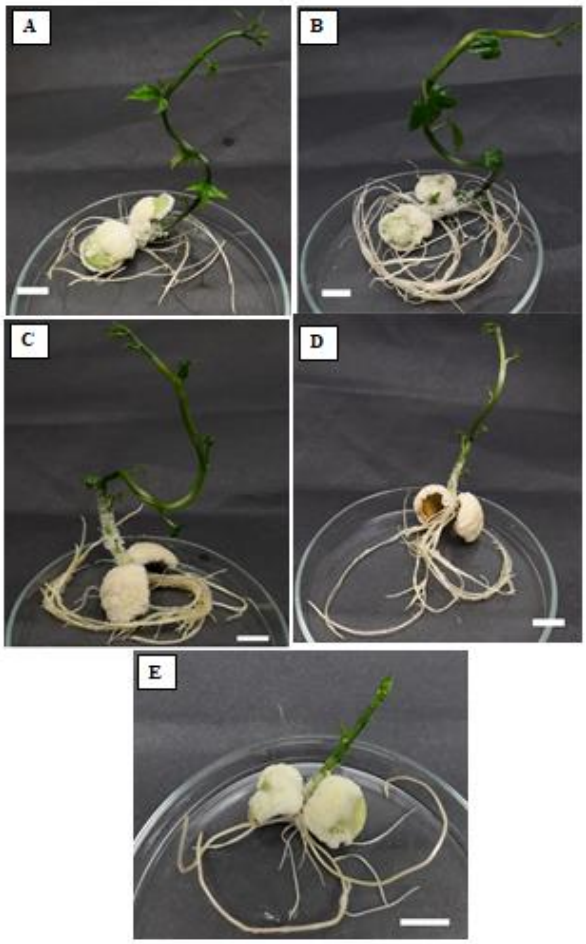

Fig 4. Effect of different LED light spectra treatments on shoot and root elongation and multiplication as well as multiplication of leaves after three weeks of culture. (A) Violet LED light treatment, (B) Blue LED light treatment, (C) Green LED light treatment, (D) Red LED light treatment and (E) Control treatment. (Scale bar = 1 cm)

$$\text{Rate of contamination (\%)} = \frac{\text{Number of contaminated seed(s)}}{\text{Total number of seeds}} \times 100\ \%$$

Establishment of seeds germination

The cultures were maintained in the growth room at a temperature of 28 ± 2 ℃ for at least one week. For light illumination treatment, the seeds were exposed to the 12 hours daylight (12 hours light/ 12hours dark). For dark treatment, the seeds were kept in an enclosed cupboard (24 hours dark). For each treatment, 30 replicates were prepared with one seed being placed on the Petri dish with MS media.

Effect of light conditions on seed germination

The seeds were observed every week (7 days) starting from the day of culture. Germinated seeds (with emerged radical) were counted and recorded. Germination rate was calculated weekly by using the following formula:

$$\text{Rate of germination (\%)} = \frac{\text{Number of germinated seed(s)}}{\text{Total number of seeds inoculated}} \times 100\ \%$$

Establishment of shoot, root, and leaf formation from seeds

Germinated seeds were transferred to MS medium containing 1 mg/L BAP and incubated in LED room at temperature of ± 25 ℃ for three weeks. Transferred seeds were cultured under different LED spectra: red, blue, violet, green, and white light as the control. Wavelengths of selected LED spectra were shown in Table 5. For each treatment, five replicates with one germinated seed each were used.

Effect of LED light spectra on shoot, root, and leaf multiplication, and root elongation

After three weeks of incubation, the number of induced shoot, root, and leaf per seed was counted and recorded while data from contaminated cultures were not included. The length of roots per seed was measured using sterile string and graph paper.

Statistical analysis

The data obtained from the experiments were analysed using IBM statistical package SPSS 24.0. The means were compared using a one-way analysis of variance (ANOVA) and differentiated through Duncan post-hoc test, with the confidence interval of 95.0 %.

Conclusions

Light facilitates gac seed germination. Light conditions were not necessary to germinate uncoated *M. cochinchinensis* seeds, but it can enhance the rate of germination with at least 12 hours photoperiod. The most effective LED light spectrum to produce the highest number of shoots, number of leaves and the average root length was green LED light at (495 – 570 nm). Violet LED light spectrum was effective in promoting the formation of the root.

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Compliance with Ethical Standards

The authors declare that they have no conflict of interest.

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