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LED Light Technology as a Source of Illumination and a Promising Method for *Stevia rebaudiana* Elite Propagation

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EFFECT of light-sources, quality, and intensities on morphogenesis, shoot multiplication, development and roots, and biomass production in *Stevia* investigated using shoot tips cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of plant growth regulators (PGRs). 2000Lux intensity provided by fluorescent light lambs was found the most favorable for shoot induction and production, shoot length, number of leaves/explant and also for fresh and dry weights/foliage. Under this light condition, the optimum contents of photosynthetic pigments, cholorophyll *a*, cholorophyll *b*, total cholorophyll, and carotenoids recorded. Blue-LED light treatment created growth condition for the highest shoot elongation, leaves number/shoot, leaf fresh weight, leaf dry weight, and photosynthetic pigment production. Cent percent of shoot induction was obtained under red-LED treatment resulting in the largest number of shoot production. Root formation was promoted by 2000 and 3000Lux intensities of light. However, the 2000Lux intensity treatment provided the most favorable growth condition permitting generation of largest number of longest roots with maximum fresh/dry weights. Root induction was 100 percent under fluorescent light, the maximum fresh and dry weights of roots were achieved on blue-Led light and red-LED treatment induced the longest roots.

Keywords: *In vitro* morphogenesis, *In vitro* rooting, Light quality, Light-emitting-diodes (LEDs), *Stevia rebaudiana*.

Introduction

Stevia rebaudiana Bertoni (Asteraceae family) is a medicinally important plant producing sweetener substances in leaves that are 100-300 times sweeter than sucrose (Ahmad et al., 2011). Stevia has been used as a natural sweetener for broad range of products in the food and soft drink industry for decades. Its leaves contain stevioside and rebaudioside (Prakash & Chaturvedula, 2018). These compounds are used for the treatment of diabetes as these are non-toxic, low-calorie, and non-mutagenic (Aman et al., 2013). The stevioside is strongly prescribed for treatment of Hypertension, depression, fatigue, and infections (Jeppensen et al., 2002; Dyrskog et al., 2005). Besides its sweet flavour, it has anti cancerous, anti hyperglycaemic, and antioxidant activities (Kinghorn & Soejarto, 1985; Starratt et al., 2002). The stevioside is also used as additive in processed foods, baking products, coffee, tea, beverages, cold drinks, and fruit juices (Deshmukh & Ade, 2012).

Stevia can be propagated through seeds (which have a poor germination rate, loose viability during storage, does not facilitate the production of homogeneous populations, leading to different of sweetening composition and concentration) as reported by Carneiro et al. (1997), Sivaram & Mukundam (2003) or using vegetative cuttings (due to the smaller number

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of individuals, needs higher input stock and laborious) as reported by Nakamura & Tamura (1985), Debnath et al. (2006), Mitra & Pal (2007). *In vitro* clonal propagation is alternative method for disease-free large-scale production in a short time and limited space (Ramirez-Mosqueda & Iglesias-Andreu, 2016; Attaya, 2017).

The important factor that influences growth, development and plant production in culture of various plant species is light (photon flux, photoperiod and spectral quality) (Gupta & Jatothu, 2013). Fluorescent lamps are the most popular light source for *in vitro* culture that range from 350 to 750 nm for promoting growth (Kim et al., 2004). A range experiments on Stevia tissue culture are carried out such as shoot apex, nodal, and leaf explants under a light intensity of 80µmol/m^{-2} s^{-1} (Sivaram & Mukondan, 2003), nodal explants under 2500Lux of light intensity (Rafiq et al., 2007). Moreover (Uddin et al., 2006; Hossain et al., 2017) used nodal segments under 2000-3000Lux of light intensity and direct leaf organogenesis was conducted at light intensity of 40µmol/$m^{-2}s^{-1}$ (Sreedhar et al., 2008). Other light sources, such as light-emitting diodes (LEDs), have proved to be more effective for *in vitro* culture due to narrow bandwidth, their wavelength specificity, low degradation, low amount of thermal emissions, and long life (Gupta & Jatothu, 2013; Ramirez-Mosqueda, 2017). Recently, few literatures have been conducted on the impact of LEDs on a wide range of physiological activities, and *in vitro* plant morphogenesis (Shin et al., 2008). These studies include wheat, spinach, pepper, lettuce, and cucumber seedlings (Bula et al., 1991; Hoenecke et al., 1992; Brown & Schuerger, 1993; Okamoto & Yanagi, 1994; Schuerger & Brown, 1994; Yanagi & Okamoto, 1994; Tripathy & Brown, 1995). Moreover, LEDs used on *in vitro* cultured potato plantlets (Miyashita et al., 1995), *in vitro* cultured *Cymbidium* plantlets (Tanaka et al., 1998), *in vitro* cultured *Rehmannia glutinosa* plantlets (Hahn et al., 2000), and *in vitro* cultured *Jatropha curcas* (Attaya & El-Sarag, 2017).

The utilization of a more versatile, efficient light source for *in vitro* plant regeneration and growth can offer new and important possibilities to achieve success in commercial micro-propagation. The present study aimed to investigate the influence of light quality and sources on *in vitro Stevia rebaudiana* propagation, focusing on the impact of new LED wavelengths technology on *in vitro* morphogenesis, shoot proliferation and root formation.

Materials and Methods

Plant material and explant sterilization

Stevia rebaudiana seeds var. spanti were collected from Sugar Crops Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt. The seeds were germinated and maintained in the greenhouse of Plant Science Department, McGill University, Canada. At plant tissue culture laboratory, Shoot tips (1.5cm long, 0.5cm wide) from Three-months-old *Stevia rebaudiana* were used as explants that cut, washed under running tap water and then submerged in tap water with a few drops of Tween-20 in a flask with shacking by hand for 5min. followed by rinsing in tap water to remove the soap. Under aseptic condition in laminar airflow hood, explants were surface sterilized with 70% (v/v) ethanol for 30sec. and subsequently with 0.15% mercuric chloride ($HgCl_2$) solution for 1 minute and washed (3 times) with sterile distilled water to remove all traces of $HgCl_2$.

Culture medium and conditions

The sterilized shoot tip explants were trimmed (0.5-1.0cm) at the base and cultured in contact with full MS basal salt mixtures including vitamins medium (Murashige & Skoog, 1962) supplemented with 30g L^{-1} sucrose and 7g L^{-1} of agar in the presence of 1.0mg L^{-1} 6-benzyladenine (BA). The pH was adjusted to 5.6-5.8 before gelling with agar and autoclaved at 121°C and 1.1kg cm^{-2} for 20min. then the media were distributed in vessels (30-40mL for each vessel). Five explants were cultured per vessel (100mL) as a replicate and the treatment was contained 5 vessels (5 replicates). For light quality experiment, the radiation intensity of artificial light was set to 40–50µmol m^{-2} s^{-1}. the cultures were maintained in an air conditioned culture room with relative humidity of 80±5% at 25±2°C under 16hrs. per day photoperiod which provided by four different cool white fluorescent lamps (light intensity 1000, 2000, 3000 and 4000Lux.) to evaluate light quality on shoot proliferation and growth. Moreover, for light sources experiment, the cultures were maintained under four different light sources i.e. fluorescent lamps as a control with wavelengths

of 4000-7000nm, white LEDs with a wavelength of 420nm, red LEDs with a wavelength of 660 nm and blue LEDs with a wavelength of 460nm) under a 16-hrs. photoperiod. (IP65 model, SMD 5050 RBG supplying 12V and 1W per module, Techno Lite®, Zapopan, Jalisco). For pigment analysis, chlorophyll and carotenoids contents were analyzed according to the method of Lichtenthaler (1987) in the third leaf counted from the top downward of plantlets. Dry weights of leaves and roots were determined after drying for 48hrs. at 70°C.

In vitro rooting and hardening

Shoots (1-2cm) in length that regenerated from *in vitro* cultured explants were rooted on half strength MS medium. The medium was supplemented with $30gL^{-1}$ sucrose and $7gL^{-1}$ agar in the presence of $1.0mgL^{-1}$ Indole butyric acid (IBA). To monitor the initiation and quality of adventitious roots on the regenerated shoots, five shoots were cultured per vessel as a replicate and the treatment was contained four vessels (4 replicates). The cultures were maintained under four different light quality (1000, 2000, 3000 and 4000Lux.) provided by cool white fluorescent lamps. Moreover, under four different light sources (fluorescent lamps, white LEDs, red LEDs and blue LEDs). Then, well-rooted shoots after 6 weeks of culture were carefully taken out from the medium and washed thoroughly with sterilized distilled water for *ex vitro* culture. The plantlets were then planted into pots (100mL) containing a mixture of organic soil and sand (1:1). Then placed in a plastic tunnel and wetted with tap water followed by covering with transparent plastic bags to maintain humidity. Watering was 3 times during the 3 weeks. The established plants were transplanted to black polyethylene bags containing garden soil and farmyard manure for further growth.

Statistical analysis

Statistical difference among the treatments means was analyzed by Duncan's multiple range test (DMRT) at 0.05 level using the SPSS (version 17) and the results were expressed as the mean± SE. Data were also subjected to analysis of variance (ANOVA).

Results and Discussion

Influence of light quality and source on in vitro shoot bud proliferation

After 6 weeks of culture, a positive influence with significant differences was observed between the different light intensities on the *in vitro Stevia rebaudiana* shoot formation (Table 1). Light intensity 2000Lux provided the most favorable growth conditions followed by 3000Lux treatment. Using 2000Lux produce 95% shoot induction, the largest number of shoots (4.42 shoots/explant), the tallest shoots (3.70cm) and the largest number of leaves (3.47 leaves/explant). Whereas 3000Lux treatment gave 3.57 shoots/explant with 3.15cm shoot length with 85% shoot induction. However, 1000Lux treatment produced shorter shoots (2.25cm) with fewer leaves (1.50), and with 70% shoot induction relative to the other treatments. Thus there was no callus formation observed between treatments in this experiment. The results are in line with Uddin et al. (2006), Hossain et al. (2017), they found that the largest number of shoots/explant, shoot length, and leaves number were produced under 2000 then 3000Lux of light intensity.

The data in Table 2 showed statistically significant variations between light intensities applied on leaf fresh weight, leaf dry weight, chlorophyll, and carotenoid contents at 5% level. treatment of 2000Lux light intensity produced the highest values of leaf fresh and dry weights (0.36g and 46.0mg/g, respectively), followed by 3000Lux treatment (0.26g and 42.5mg/g, respectively). Meanwhile, 1000Lux treatment produced the fewer values of leaf fresh and dry weights (0.15g and 39.2mg/g, respectively). Also, the higher contents of cholorophyll *a*, cholorophyll *b*, total cholorophyll and carotenoids were produced under 2000Lux that shows the best values (1.55, 3.07, 4.62 and 0.06mg/g respectively), followed by 3000Lux that exhibits (1.32, 2.32, 3.65 and 0.05mg/g, respectively). The treatment of 1000Lux of light intensity gave the lowest values of all studied traits.

A positive influence with significant variations between the fluorescent and LEDs was recorded on the *in vitro Stevia rebaudiana* shoot formation (Table 3, Fig. 1) after 6 weeks of culture. The largest number of shoots (6.65 shoots/explant) with 100% shoot induction was obtained under Red-LED treatment, followed by Blue-Led that gave 5.47 shoots/explant with 95% shoot induction.

TABLE 1. Influence of light quality on shoot induction and production of *in vitro* Stevia explants after 6 weeks

Light intensity (Lux)	Shoot induction%	No. of shoots/ explant	Length of shoot (cm)	No. of leaves/ explant	Callus formation
1000	70^{d}	2.30±0.15^{c}	2.25±0.08^{c}	1.50±0.08^{d}	No
2000	95^{a}	4.42±0.08^{a}	3.70±0.05^{a}	3.47±0.08^{a}	No
3000	85^{b}	3.57±0.16^{b}	3.15±0.08^{b}	3.10±0.10^{b}	No
4000	75^{c}	2.60±0.12^{c}	2.42±0.08^{c}	2.10±0.12^{c}	No

According to the Dunchans multiple range test (DMRT) at 0.5 level, the means± SE (standard error) in each column followed by the same letters are not significantly different.

TABLE 2. Influence of light quality on leaf fresh weight, leaf dry weight, chlorophyll and carotenoid contents of *in vitro* Stevia explants after 6 weeks

Light intensity (Lux)	Leaf fresh weight (g)	Leaf dry weight (mg g^{-1} FW)	Chlorophyll (mg g^{-1} FW)			Carotenoids (mg g^{-1} FW)
			a	*b*	Total	
1000	0.15±0.00^{d}	39.25±0.47^{d}	0.77^{d}	1.47^{d}	2.25±0.10^{d}	0.03^{d}
2000	0.36±0.01^{a}	46.00±0.40^{a}	1.55^{a}	3.07^{a}	4.62±0.08^{a}	0.06^{a}
3000	0.26±0.00^{b}	42.50±0.28^{b}	1.32^{b}	2.32^{b}	3.65±0.06^{b}	0.05^{b}
4000	0.17±0.01^{c}	40.50±0.28^{c}	1.10^{c}	1.65^{c}	2.75±0.09^{c}	0.04^{c}

According to the Dunchans multiple range test (DMRT) at 0.5 level, the means ± SE (standard error) in each column followed by the same letters are not significantly different.

TABLE 3. Influence of light source on shoot induction and production of *in vitro* Stevia explants after 6 weeks

Light source	Shoot induction %	No. of shoots/ explant	Length of shoot (cm)	No. of leaves/ explant	Callus formation
Fluorescent	90^{c}	4.35±0.09^{d}	3.82±0.16^{a}	3.65±0.14^{c}	No
Red-LED	100^{a}	6.65±0.15^{a}	3.02±0.19^{b}	2.82±0.14^{d}	No
Blue-LED	95^{b}	5.47±0.16^{b}	3.67±0.18^{a}	5.27±0.12^{a}	No
White-LED	90^{c}	4.85±0.10^{c}	3.57±0.18^{ab}	4.52±0.17^{b}	No

According to the Dunchans multiple range test (DMRT) at 0.5 level, the means ± SE (standard error) in each column followed by the same letters are not significantly different.

Fig. 1. Influence of light source on *Stevia rebaudiana* morphogenic responses after 6 weeks of culture

Then White-LED treatment which produce 4.85 shoots/explant with 90% shoot induction. However, the tallest shoot 3.67 cm with largest number of leaves 5.27 was obtained under Blue-Led treatment. On the other hand, Red-LED treatment produced shorter shoots (3.02cm) with fewer leaves (2.82) in comparison to other LED treatments. Using fluorescent lambs as a control treatment produced the fewest number of shoots (4.35) and longer shoots (3.82cm) per explant. Using LEDs light technology by red, blue or white sources leads to have great values on shoot proliferation and growth than fluorescent lamps. These findings are in line with Ramirez-Mosqueda et al. (2017), who found that Red-LEDs produce a larger number of shoots/explant comparing to white or blue-LEDs. Also, Hahn et al. (2000) found that shoot lengths under either blue or red LED were greater than under fluorescent lamps. In addition, the findings are in harmony with Macedo et al. (2011) who stated that Blue-LED produce a larger number of leaves per shoot on *Alternanthera brasiliana*.

The data in Table 4 were revealed statistically significant differences between the fluorescent and different LEDs on leaf fresh weight, leaf dry weight, chlorophyll, and carotenoid contents at 5% level. Blue-LED light treatment provided the most favorable growth conditions inducing the best values of leaf fresh and dry weights (0.61g and 46.7mg/g, respectively), followed by White-LED light treatment recorded the second best values of leaf fresh and dry weights (0.51g and 44.5mg/g, respectively). Then, Red-LED light treatment was in the third level that showed (0.38g and 43.5mg/g, respectively). However, fluorescent light treatment produced the fewer value of leaf fresh weight (0.35g) with high dry weight (46.2mg/g). In addition, the higher contents of cholorophyll *a*, cholorophyll *b*, total cholorophyll, and carotenoids were obtained under Blue-LED light treatment that showed the best values of (12.2, 6.5, 18.7 and 2.5mg/g respectively), followed by White-LED light treatment that showed the values of (8.0, 6.0, 14.1 and 1.2mg/g, respectively). The fluorescent light treatment gave the lowest values of all studied traits. These findings are in line with Macedo et al. (2011) who stated that blue-LED produced a larger number of leaves per shoot on *Alternanthera brasiliana*. In contrast, leaf biosynthesis pigments of *Doritaenopsis* hort. (Orchidaceae) plants were substantially higher under red plus blue LEDs than under red or blue LEDs or under fluorescent light (Shin et al., 2008).

Influence of light quality and source on in vitro root formation

A positive influence with significant differences was observed between the different light intensities on the *in vitro Stevia rebaudiana* root formation (Table 5), After 6 weeks of culture. Treatments with 2000 and 3000Lux showed 100% root formation followed by 4000Lux treatment that obtained 95% root formation. treatment 2000Lux of light intensity provided the most favorable growth condition which produced the largest number of roots (6.55 roots/shoot) with the tallest roots (2.55cm), and with the largest root fresh and dry weights (1.52 g, 68.2mg/g, respectively), followed by 3000Lux treatment that observed (6.05 roots/shoot) with (2.20cm root length), and (1.42g, 67.0mg/g, respectively) of root fresh and dry weights. However, 1000Lux treatment produced shorter roots (1.45cm) with fewer root fresh and dry weights (1.02g, 62.0mg/g, respectively), and with 85% root induction relative to the other treatments. The results are in line with Uddin et al. (2006), Hossain et al. (2017) who found that the largest number of roots/shoot explant, and root length were produced under 2000 then 3000Lux of light intensity.

TABLE 4. Influence of light source on leaf fresh weight, leaf dry weight, chlorophyll and carotenoid contents of *in vitro* Stevia explants after 6 weeks

Light source	Leaf fresh weight (g)	Leaf dry weight (mg g^{-1} FW)	Chlorophyll (mg g^{-1} FW)			Carotenoids (mg g^{-1} FW)
			a	*b*	Total	
Fluorescent	0.35±0.00^{d}	46.25±0.47^{a}	1.67^{d}	3.10^{c}	4.77±0.06^{d}	0.06^{d}
Red-LED	0.38±0.00^{c}	43.50±0.28^{b}	5.05^{c}	6.45^{a}	11.50±0.04^{c}	0.71^{c}
Blue-LED	0.61±0.01^{a}	46.75±0.25^{a}	12.2^{a}	6.52^{a}	18.72±0.03^{a}	2.50^{a}
White-LED	0.51±0.00^{b}	44.50±0.64^{b}	8.07^{b}	6.07^{b}	14.10±0.06^{b}	1.22^{b}

According to the Dunchans multiple range test (DMRT) at 0.5 level, the means ± SE (standard error) in each column followed by the same letters are not significantly different.

TABLE 5. Influence of light quality on adventitious root formation of *in vitro* Stevia shoots after 6 weeks

Light intensity (Lux)	Root formation %	Roots no./shoot	Root length (cm)	Fresh weight of roots (g)	Dry weight of roots (mg g^{-1} FW)
1000	85[c]	4.50±0.08[d]	1.45±0.06[d]	1.02±0.02[c]	62.0±0.70[b]
2000	100[a]	6.55±0.12[a]	2.55±0.06[a]	1.52±0.05[a]	68.2±0.49[a]
3000	100[a]	6.05±0.05[b]	2.20±0.09[b]	1.42±0.02[a]	67.0±0.91[a]
4000	95[b]	5.50±0.08[c]	1.97±0.06[c]	1.22±0.05[b]	64.0±0.41[b]

According to the Dunchans multiple range test (DMRT) at 0.5 level, the means ± SE (standard error) in each column followed by the same letters are not significantly different.

Data shown in Table 6 and Fig. 2 were revealed statistically significant differences between the fluorescent and LEDs on *Stevia rebaudiana* root formation. The largest roots number (6.55 roots/shoot) with 100% root induction was produced under fluorescent light condition, followed by blue-Led, that gave (5.17 roots/shoot) with 85% root induction. Then red-LED treatment which produced (4.25 roots/shoot) with 90% root induction. Moreover, the longest root 3.07 cm was observed under red-Led treatment. On the other hand, white-LED treatment produced shorter roots (1.62 cm) relative to the other LED treatments (Fig. 2). For root fresh and dry weights, blue-Led treatment produced the largest values of 1.55g and 68.2mg/g, respectively, followed by white-LED (1.42g and 65.5mg/g), then fluorescent light treatment that induced (1.32g and 64.5mg/g). The red-LED light treatment showed the lowest values of root fresh and dry weights (1.10g and 63.5mg/g), respectively. The results indicated that the different LED light sources enhanced the Stevia *in vitro* rooting. These findings are in harmony with Shin et al. (2008), Li et al. (2013) and Lim & Eom (2013) who reported that LEDs increase the number and length of roots/shoot especially blue-LEDs in different *in vitro* plant species. Moreover, Hahn et al. (2000) found that root lengths under either blue or red LED were greater than under fluorescent lamps. On contrast, Ramirez-Mosqueda et al. (2017), found that red + blue LEDs produced a larger number of roots/shoot explant comparing to red, blue or white-LEDs.

Acclimatization and field transfer

The survival rate of *in vitro* propagated plantlets was 70-90% depending on activated type of light as fluorescent and different LED light conditions. The highest rate (90%) was occurred with fluorescent light treatment, followed by (70%) that observed with blue-LED treatment, red-LED, and white-LED. Finally, the plantlets grew very well in the greenhouse and ready for field transfer (Fig. 3).

Conclusion

In the present study an efficient and highly reproducible protocols has been made to get healthy *Stevia rebaudiana* plants in a relatively short period and with a high survival rate. In addition, high-quality Stevia plants can be grown *in vitro* under various blue, red, and white LEDs as a new insight for mass propagation and genetic improvement.

TABLE 6. Influence of light source on adventitious root formation of *in vitro* Stevia shoots after 6 weeks

Light source	Root formation%	Roots no./shoot	Root length (cm)	Fresh weight of roots (g)	Dry weight of roots (mg g^{-1} FW)
Fluorescent	100[a]	6.55±0.13[a]	2.57±0.08[b]	1.32±0.02[b]	64.50±0.29[c]
Red-LED	90[b]	4.25±0.13[c]	3.07±0.08[a]	1.10±0.07[c]	63.50±0.29[d]
Blue-LED	85[c]	5.17±0.15[b]	2.22±0.05[c]	1.55±0.03[a]	68.25±0.25[a]
White-LED	75[d]	2.65±0.13[d]	1.62±0.11[d]	1.42±0.02[ab]	65.50±0.29[b]

According to the Dunchans multiple range test (DMRT) at 0.5 level, the means ± SE (standard error) in each column followed by the same letters are not significantly different.

Fig. 2. Influence of light source on *Stevia rebaudiana* adventitious root formation after 6 weeks of culture

Fig. 3. *In vitro* propagated Stevia plantlet ready for field transfer

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تقنية الإضاءة الحديثة باستخدام لمبات الليد كمصدر للإضاءة وطريقة واعده لإكثار الاستيفيا معملياً

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تركز هذه الدراسة على دراسة تأثير جودة الإضاءة باستخدام لمبات الفلوريسنت بمستويات شدة اضاءة مختلفة وكذلك مصادر الإضاءة الحديثة باستخدام لمبات الليد وتأثيرها على مورفولوجيا ونمو وتطور وتجذير نبات الاستيفيا معملياً لإكثارها معملياً وللحصول على نباتات صحية وذات معدل انتاج وفير باستخدام القمم النامية وبعض منظمات النمو النباتية المختلفة على بيئة موراشيجي وسكوج.

أوضحت النتائج ان استخدام لمبات الفلوريسنت ذات مستوى شدة اضاءة 2000 شمعة وبعد 6 أسابيع من الزراعة انها الأفضل لنمو الأجزاء الخضرية حيث أعطت اعلى القيم لصفات تكشف البرعم خضري وعدد البراعم المستنبته للمنفصل النباتي الواحد وطول البرعم وعدد الأوراق للمنفصل النباتي والوزن الغض والجاف للأوراق. كذلك أعطت اعلى النتائج لمحتوى الكلوروفيل أ والكلوروفيل ب والكلروتينات لعملية البناء الضوئي.

كذلك فان هذه الدراسة تقترح انه يمكن اكثار وإنتاج نباتات استيفيا جيدة معمليا تحت ظروف اضاءة باستخدام لمبات الليد الزرقاء والحمراء والبيضاء والتي تؤدي للحصول على نتائج جيدة لنمو وتكشف الأجزاء الخضرية عن لمبات الفلوريسنت كرؤية جديدة للإكثار الدقيق. أظهرت معاملة الإضاءة باستخدام لمبات الليد الزرقاء انها الافضل لاستطالة البراعم الخضرية حيث أعطت اعلى النتائج لصفات طول البرعم وعدد الأوراق للجزء النباتي والوزن الغض والجاف للأوراق وكذلك محتواها من الكلوروفيل أ والكلوروفيل ب والكاروتينات بالرغم من ان اعلى عدد للبراعم وتكشفها بنسبة 100% ظهر طبقاً لمعاملة الاضاءة باستخدام لمبات الليد الحمراء.

خلال مرحلة التجذير معملياً أظهرت مستويات شدة الإضاءة 2000 و3000 شمعة باستخدام لمبات الفلوريسنت اعلى معدل تجذير بنسبة 100% وكذلك فإن شدة الإضاءة 2000 شمعة كانت الأفضل لنمو الأجزاء الخضرية والتي أدت للحصول على اعلى القيم لصفات عدد الجذور واستطالة الجذور والوزن الغض والجاف للجذور. بالنسبة لتأثير مصادر الإضاءة المختلفة كان اعلى معدل للتجذير بنسبة 100% وأكبر عدد جذور للمنفصل النباتي تم الحصول عليهما باستخدام لمبات الفلوريسنت في الإضاءة ثم بعد ذلك استخدام لمبات الليد الزرقاء والتي أعطت اعلى القيم لصفات الوزن الغض والجاف للجذور ثم استخدام لمبات الليد الحمراء التي أعطت اعلى معدل استطالة للجذور. بالإضافة الى ان الشتلات الصحية المتطورة والتي تم الحصول عليها معملياً تمت اقلمتها بنجاح بعد 6 أسابيع واخذت تنمو طبيعياً في الصوبة الزجاجية.