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EFFECTS OF LIGHT INTENSITY ON *IN VITRO* REGENERATION OF *STEVIA REBAUDIANA* BERTONI, *BACOPA MONNIERI* L. AND *SOLANUM TUBEROSUM* L.

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Abstract

Light is the most essential factor controlling plant growth, morphogenesis, metabolism and chlorophyll content of plant cells, tissues and organ culture. The present investigations were carried out to find out effects of different light intensities (210, 500, 1000, 1500, 2000, 2500 and 3000 lx) on the regeneration of three plants, namely *Stevia rebaudiana* Bertoni., *Solanum tuberosum* L. and *Bacopa monnieri* L. Results showed that light intensity has a direct effect on plant growth *in vitro*. There was less time for shoot initiation and the highest mean number of shoots/explants (9.75 ± 3.862) of *S. rebaudiana* was observed from the nodal explants when the culture was maintained at 1000 lx light conditions. At maximum light intensity (3000 lx) best result was recorded for *S. tuberosum*. A distinct intensity of light, 1500 lx showed the best outcome both for the number and length of shoots of *B. monnieri* ($p = 0.00$). The present study also demonstrated the relationship between root growth and light condition.

Introduction

In natural light conditions, plants produce more carbohydrates for use and storage, whereas plants inside the laboratory and green house, the physical structure shades the plants and induces a light deficit (Pati 2014). In recent years fluorescent lamps (FLs), high pressure sodium, metal halide and incandescent lamps have been commonly used in tissue culture growth rooms as artificial light sources, which are energy and cost-effective (Okwuonu *et al*. 2017). Despite the fact that light emitting diodes (LEDs) can specify wavelength and consume less energy, they require special installation, so white fluorescent lamps remain popular as a light source for commercial micropropagation. For the advanced running of a functional tissue culture laboratory, there was a need to explore lighting effects on tissue cultured plants. Considering this lighting effect on micropropagation, three rapidly propagated plants like *Stevia rebaudiana* Bertoni., *Solanum tuberosum* L. and *Bacopa monnieri* L. were used to evaluate the effect of light on those plants. *S. rebaudiana*, *B. monnieri* both important medicinal plants and *S. tuberosum* is an important vegetable tuber crop. Thus the present study was aimed to find the effect of light intensity under different lighting conditions using fluorescent lamps (FLs) of lux meter on *in vitro* morphogenic processes following shoot and root formation of *S. rebaudiana* Bertoni, *B. monnieri* and *S. tuberosum* var. diamant.

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Materials and Methods

In vitro regenerated mother plants of *Stevia rebaudiana* Bertoni, *Bacopa monnieri* L. and *Solanum tuberosum* L. var. diamant. were maintained in the tissue culture laboratory of BCSIR (Bangladesh Council of Scientific and Industrial Research). Nodal segment explants were collected from the *in vitro* grown plantlets of the three studied plants and sub-cultured on MS (Murashige and Skoog 1962) basal medium with various auxin supplemented with 0.8% agar and 3% sucrose. The cultures were maintained for 60 days in a growth chamber at 25 ± 2°C air temperature and at 16/8 h light/dark photoperiod with various light intensity. The Lux Meter (Digital Lux Meter, Model: LX 1010B, China) is a device to measure light intensity (SI unit: lux, Symbol: lx) per unit area supplied by cool tubular white fluorescent lamps (LM-36W/T8, Day light, BDS-292, 22 v-50 hz, 12000 mm, 6500 k). Different culture racks contained different light intensities, and each rack was covered with black paper so that each rack had the targeted light intensity. Regenerated shoots were inoculated in root induction media and maintained on dark and light conditions to compare the effects of light and dark on root formation and development. The experiment was arranged in a completely randomized design (CRD) where each treatment combination was replicated three times. The Duncan Multiple Range Test (DMRT) has been performed at a 5% level of significance both for shoot number and shoot length. All statistical analysis were performed by statistical software, IBM SPSS Statistics V22.0.

Results and Discussion

The present experiment revealed that light of different intensities, influenced plant growth and had a significant effect on plant regeneration and elongation. In the case of *S. rebaudiana,* less time (6-7 days) was required for shoot initiation when nodal explants were incubated at 1000 lx and 1500 lx light intensities (Fig. 1a). Moreover, multiple shoot formation was not similar in both the light conditions (1000 lx and 1500 lx). The highest mean number (9.75 ± 3.862) of shoots/explant was observed from the nodal explants when the culture was maintained at 1000 lx light conditions (Fig. 1b, and Table 1). The intensity of 1000 lx maximum mean length (4.70 ± 0.903 cm) of shoots per explant was also recorded (Fig.1c). On the other hand, it was observed that the value of the mean number of shoots/explant was reduced at 1500 lx but suddenly the value was increased at 2000 lx intensity (Table 1). Modi *et al.* (2012) reported 5.5 shoots from nodal explants of *S. rebaudiana* using fluorescent-lamp light on modified MS medium and 1 % (w/v) sucrose without any plant growth regulators. The present experiment revealed that at more than 2000 lx and less than 500 lx, the *S. rebaudiana* plant produced a comparatively lower number of multiple shoots compared to other light intensities. According to DMRT, no significant trend of shoot number of *S. rebaudiana* has been noticed for different levels of light intensity ($p=0.104>0.05$), but for length of shoots, light of 1000 lx shows the highest effect ($p=0.018<0.05$). In *Phoenix dactylifera* L., high intensities (2000-3000 lx) decreased shoot bud proliferation and stimulated shoot elongation and greening (Meziani *et al.* 2015). In contrast, the present study showed that when exposed to 1000 lx of light, the Stevia leaf grew larger and stimulated shoot elongation (Fig. 1c). The result of the effect of different light intensities (210-3000) lx on the *in vitro* growth of *S. rebaudiana* is presented in Table 1. As seen from Table 1, increasing light intensity and *in vitro* growth of *S. tuberosum* are directly related. Growth, morphogenesis, and tuberization of potato tissues *in vitro* are affected by light (Seabrook 2015). The current experiments revealed that a light intensity of 3000 lx stimulates regeneration the most. At 3000 lx intensity, 4-5 days were required for the initiation of shoots (Fig. 1d) and multiple shoots were regenerated within 8-9 days. The highest mean number of shoots (6.33 ± 1.211) and height (7.72 ± 1.679 cm) were recorded at this light intensity (Fig.1e and Table 1). At 2500 lx intensity *S. tuberosum* plants also showed the good regeneration response with 6.5 ± 1.049 mean number of

shoots and 5.633 ± 0.744 cm average heights. Table 1 revealed that poor health and less number of multiple shoots were regenerated at 210 and 500 lx light intensity. Rehana *et al.* (2018) observed the effect of average light intensity at sunlight and artificial florescence light treatment at 4805.5 ± 326.54 lx and 3484 ± 84.44 lx, respectively towards *in vitro* growth of *S. tuberosum*. They mentioned that all the growth factors performed better result in sunlight treatment than those of artificial one except average number of nodes and leaves. In the present experiment it was noticed that at high light intensity (3000 - 2500 lx) leaves of *S. tuberosum* became larger and thicker compared to other light intensities (Fig. 1h). High light intensity substantially increased the total number of expanded leaves, dry matter, sugar content and nitrogen absorbed in *Phalenopsis* (Kubota and Yoneda 1993). On the other hand, Ramírez-Mosqueda *et al.* (2017) reported that the

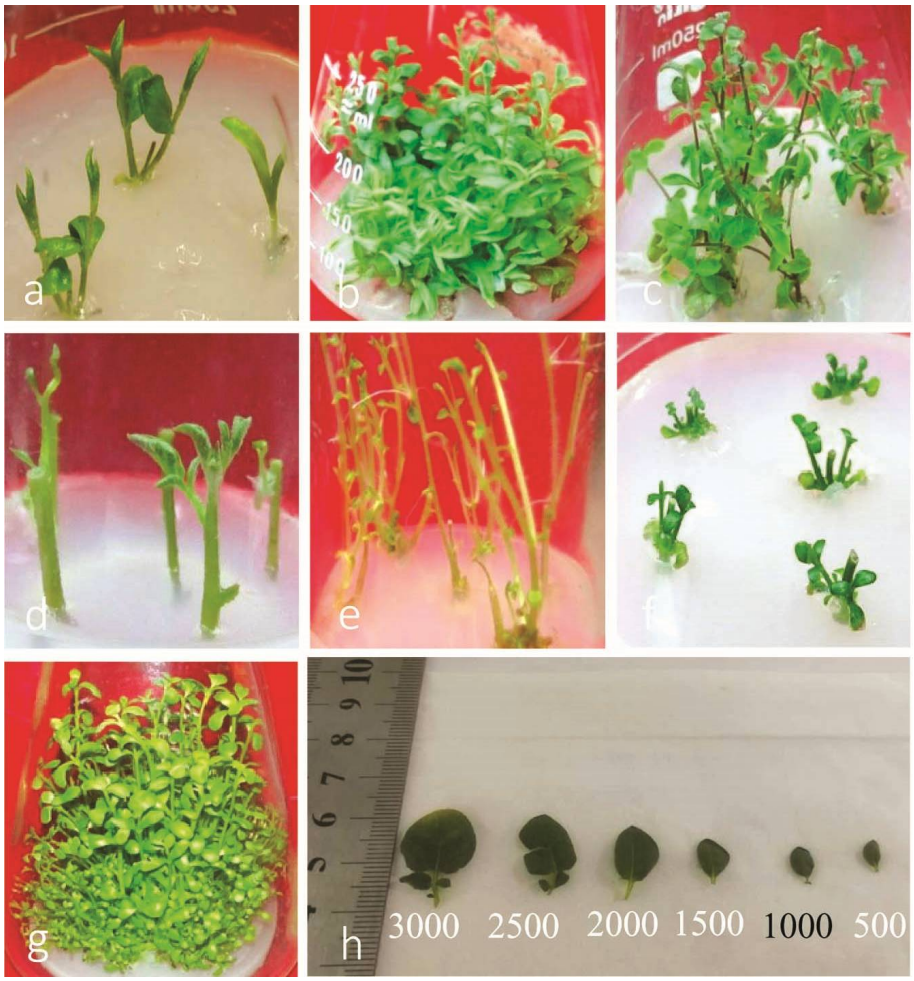

Fig.1. Effect of light intensity towards shoot regeneration from studied plants species. (a) Shoot initiation of *S. rebaudiana* on 1500 lx light intensity; (b) Formation of multiple shoots on 1000 lx light condition; (c) Maximum mean length (4.70 ± 0.903 cm) of shoots of *stevia* on 1000 lx light intensity; (d) Shoot initiation on 3000 lx from nodal explant of *S. tuberosum*; (e)) Formation of multiple shoots of *Solanum* on light as mention in fig d; (f) Formation of shoots on 1000 lx from nodal explant of *B. monnieri;* (g) Elongated multiple shoots of B. *monnieri* on 1500 lx light intensity; (h) Change of leaf size in various light intensities.

proliferation rate of *S. tuberosum* was higher with red LEDs compared with fluorescent lamp light, although shoots have a lower length under red LEDs. In contrast, Johkan *et al.* (2010) reported that lighting with blue and red LEDs and white fluorescent light did not influence the number of lettuce leaves.

Table 1. Effects of different light intensity on *Stevia rebaudiana, Solanum tuberosum* and *Bacopa monnieri.*

Light intensity (lux)	Name of plant	Days required for initiation of shoots	Mean no. shoots/explant after 60 days.	Mean length of shoots/explant (cm) after 60 days
	Stevia rebaudiana	14-15	5.80 ± 1.304 ^{b}	2.65 ± 0.659 ^{b}
210	*Solanum tuberosum*	11-12	3.25 ± .957 ^{e}	2.92 ± 0.435 ^{d}
	Bacopa monnieri	18-20	17.5 ± 1.915 ^{c}	4.88 ± 0.635 ^{b}
500	*Stevia rebaudiana*	10-11	8.80 ± 2.775 ^{ab}	3.33 ± 1.075 ^{b}
	Solanum tuberosum	7-8	3.86 ± 1.169 ^{de}	3.90 ± 0.542 ^{cd}
	Bacopa monnieri	5-6	21.75 ± 4.856 ^{bc}	6.467 ± 1.579 ^{b}
1000	*Stevia rebaudiana*	6-7	9.75 ± 3.862 ^{a}	4.70 ± 0.903 ^{a}
	Solanum tuberosum	7-8	4.13 ± 0.991 ^{de}	3.89 ± 0.803 ^{cd}
	Bacopa monnieri	6-7	28.33 ± 7.024 ^{b}	5.52 ± 1.75 ^{b}
1500	*Stevia rebaudiana*	6-7	7.25 ± 1.708 ^{ab}	3.22 ± 0.247 ^{b}
	Solanum tuberosum	6-7	4.83 ± .753 ^{cd}	4.02 ± 0.637 ^{cd}
	Bacopa monnieri	5-7	37.66 ± 3.055 ^{a}	14.8 ± 0.416 ^{a}
2000	*Stevia rebaudiana*	13-14	9.50 ± 3.697 ^{ab}	3.322 ± .400 ^{b}
	Solanum tuberosum	6-7	5.5 ± 1.049 ^{bc}	4.83 ± 0.692 ^{bc}
	Bacopa monnieri	8-9	29 ± 3.651 ^{b}	5.23 ± 1.73 ^{b}
2500	*Stevia rebaudiana*	13-14	6.40 ± 1.517 ^{ab}	2.94 ± 0.792 ^{b}
	Solanum tuberosum	4-5	5.5 ± 1.049 ^{ab}	5.633 ± 0.744 ^{b}
	Bacopa monnieri	11-14	24.6 ± 7.701 ^{bc}	6.85 ± 1.462 ^{b}
3000	*Stevia rebaudiana*	13-14	6.40 ± 1.342 ^{ab}	2.942 ± 0.792 ^{b}
	Solanum tuberosum	4-5	6.33 ± 1.211 ^{a}	7.72 ± 1.679 ^{a}
	Bacopa monnieri	14-15	22.25 ± 3.403 ^{bc}	7.49 ± 2.546 ^{b}

The different letters indicate significant differences between means of treatments according to DMRT at 5% level of significance

Explants of *B. monnieri* produced a significant number of shoots across the entire light intensity range (210-3000 lx) (Table 1, Fig. 1f). A distinct intensity of light, 1500 lx, shows the best outcome both for the number and length of shoots of *B. monnieri* ($p = 0.00$). The maximum number of (37.66 ± 3.055) shoots/explant and tremendous shoot length (14.8 ± 0.416 cm) were recorded after 45 days of culture at 1500 lx light intensity (Fig. 1g). Though comparatively less time (5-6 days) was required for the initiation of shoots at 500 lx intensity. But the mean shoot number and length were not up to the mark in this light. A considerable number of multiple shoots were found to be produced from the explants when they were maintained at 1000 as well as 2000 lx light intensities. In 1500 lx light, shoot height was almost doubled compared to other ranges of light intensity (Table 1). According to Karatas *et al.* (2016) a white LED lighting system was found to be more effective for regenerating the maximum number of (26.11) shoots per explant on the upper half of the leaves of *B. monnieri.*

Various types of auxin, such as IAA, IBA and NAA with half and full strength of MS media were applied for induction of roots in the standard 16/8 lighting condition of the growth room. Results of the experiment demonstrated that roots were induced more or less in all three types of auxin (Fig. 2). In the case of *S. rebaudiana* and *S. tuberosum*, the highest mean number of roots was induced on half strength MS medium supplemented with 2.4 µM IBA (Fig. 2). On the other hand, for *B. monnieri*, the highest root formation was observed on the full strength of MS medium supplemented with 2.6 µM NAA (Fig. 2). Result regarding the formation of roots of *B. monnieri* is contrary to the results of Mehta *et al.* (2012), Begum and Mathur (2014). They mentioned that root bud induction from *in vitro* generated shoots of *B. monnieri* was highest on ¼ strength of MS medium supplemented with 2.0 mg/l IBA. This difference might be due to the variation in environmental conditions of the plants. Kumar *et al.* (2016), reported the same results as mentioned in the present study in *S. rebaudiana*. *In vitro* shoots of *S. tuberosum* treated with 0.5 mg/l of IBA produced roots profusely within 21 days (Molla *et al.* 2011).

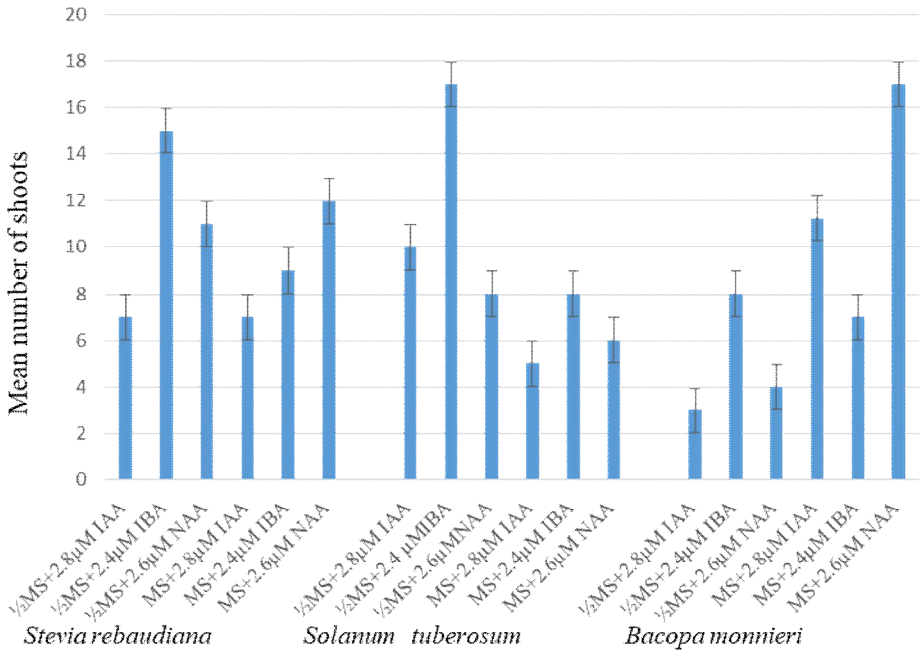

Fig. 2. Effects of different concentrations of growth hormones on half and full strength of MS medium for root induction from *in vitro* regenerated shoots of *S. rebaudiana*, *S. tuberosum* and *B. monnieri*.

An experiment was conducted using *in vitro* generated shoots towards the formation of roots in the dark as well as standard 16/8 hrs lighting conditions (Table 2). In dark conditions, *in vitro* generated shoots were inoculated on a root induction media which was covered with black paper and aluminum foil (Fig. 3a). It was clearly observed that *S. tuberosum* and *B. monnieri* have direct effect on root elongation in the absence of light, though the initiation of root was more or less the same in both conditions (Table 2). Both the plants showed maximum root length in dark conditions (Fig. 3b, 3c and 3d). Similar findings were obtained from *in vitro* rooting of micropropagated shoots from mature and juvenile *Acacia mangium* (Monteuuis and Bon 2000). Additionally, they mentioned that on an auxin supplemented medium in darkness resulted in a greater number of adventitious roots being formed than under the standard 16/8 hrs lighting

conditions. The reason for this enhancement could be that natural auxin levels increase in the dark, and CKs synthesis is stimulated in red light wavelengths (George *et al*. 2008). Meziani *et al.* (2015) and Afshari *et al*. (2011) also showed that culturing explants in the darkness significantly increased the number of formed roots in date palm culture as well as various rapeseed genotypes, respectively. On the other hand, under the standard 16/8 hrs lighting conditions, *Stevia* significantly increased the initiation of root formation rather than dark conditions (Fig. 3e, Table 2). According to Ramirez-Mosqueda *et al.* (2017) in *S. rebaudiana*, fluorescent light produced more roots per explant (12.30) and a higher length (2.13 cm), contrasting with the results observed with the different types of LEDs. They also mentioned that the different LEDs did not enhance the *in vitro* rooting of *S. rebaudiana.*

Table 2. Effects of light condition on formation and length of root.

Plant	Light condition			Dark condition		
	Responsive shoots for root formation (%)	Days required for root initiation	Mean length (cm)	Responsive shoots (%)	Days required for root initiation	Mean length (cm)
S. rebaudiana	87.5	13-16	0.55±0.17	62.25	16-20	0.4±0.08
S. tuberosum	90	8-12	2.77±0.19	90	8-12	4.5±0.36
B. monnieri	56.25	12-16	2.95±0.19,	56.25	12-16	3.25±1.3

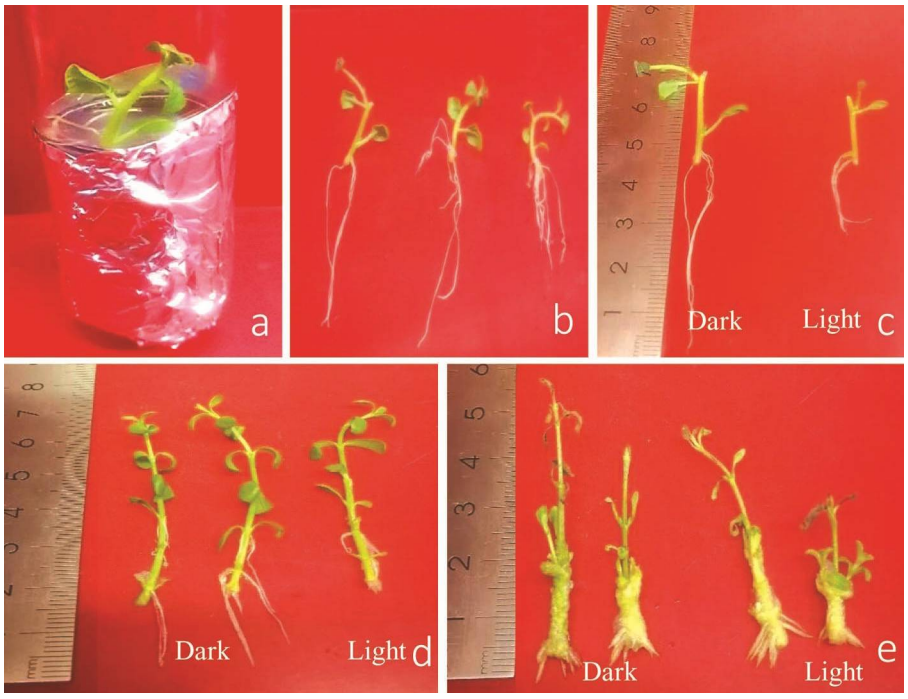

Fig. 3. Formation of roots in dark and light condition of plants species. a. Shoot in *S. tuberosum* dark rooting condition; b. Formation of roots of *S. tuberosum* on dark condition. c. Roots of *S. tuberosum* on dark and light condition. d. Roots of *B. monnieri* on dark as well as light condition. e. Same as fig d. but in *S. rebaudiana.*

The present investigation was conducted scientifically to characterize effects of light by demonstrating that light intensity is an important parameter for understanding photo morphogenesis in plant tissue culture. The present research work was carried out with the hope of getting a better understanding of how plants cope with changing conditions, especially during these global warming issues.

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