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# EFFECT OF LIGHT AND DARK ON CALLUS INDUCTION AND REGENERATION IN TOBACCO (*NICOTIANA TABACUM* L.)

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*Key words*: Organogenesis, Light effect, Regeneration, Tobacco

**Abstract**

To determine the effect of light and dark on callus induction and plant regeneration from five different explants of three tobacco varieties were studied. Normal white light (3000 lux) and dark conditions were considered for callus induction. Among the explants earliest callusing initiated at 5$^{th}$ day from nodal segment and the highest amount of callus (97.20%) was recorded from leaf blade of Motihari variety in light condition. The lowest callusing was recorded 24.40% in dark condition for root of Virginia variety and it was initiated after 13 days of culture initiation. The calli developed in dark showed watery, glossy silver color and less embryogenic features. The highest plant regeneration (95.56%) was recorded from nodal segment in light which was around five times higher than root in dark for Motihari variety. Nodal segments that received light (treatment) showed maximum relative growth rate (4.58) for Motihari variety while the lowest value was 0.91 scored from root of Virginia variety which received dark treatment.

## Introduction

Commonly callus is induced either in absence or presence of light. In many plants, such as barley (Haque and Islam 2014), *Vicia faba* (Almaghrabi 2014), maize (Morshed *et al.* 2014, Pathi *et al.* 2013), wheat (Islam 2010) and taro (Paul *et al.* 2014) callus is produced in dark; while in tomato (Sherkar and Chavan 2014), tobacco (Yanjie 2004), chilli (Kumar *et al*. 2010) in light condition. To regenerate healthy plants, there is no alternative of light which related to make the plants autotrophic. In dark and light, calli initiated, while the morphological and physiological features of induced calli were different in both conditions in tobacco (Yanjie 2004). Afshari *et al.* (2011) expressed that light stimulates the growth of calli in *Brassica.* Light of certain qualities is known to function in regulation of plant growth and development (Vince 1964). So far as we know, till now there are not enough reports on the effect of white light and dark to callus induction and plant regeneration in respect of physiological and morphological development in tobacco. Hence, the present study was undertaken to observe the effect of light and dark on callus induction, plant regeneration, related morphology of calli and its growth and development using some tobacco varieties of Bangladesh.

## Materials and Methods

Matured seeds of three tobacco varieties *viz.* Motihari, Jati and Virginia were collected from local farmers of the greater Rangpur district of Bangladesh. Seeds were washed by sterile distilled water for 6 - 7 times and deep in a 15 ml sterile falcon tube with sterilized water for 15 minutes. The floating seeds were discarded and fresh were sterilized with 70% (v/v) ethanol for two minutes and 0.1% mercuric chloride for five minutes (Ekrum 2001). To produce *in vitro* seedling, the sterilized seeds were inoculated into culture bottle containing MS (Murashige and Skoog

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1962) basal medium. The seedlings age of 25 - 30 days (d) were used as the source of explants i.e. root (R), internodal segment (INS), nodal segment (NS), leaf petiole (LP) and leaf blade (LB).

Approximately 1 sq. cm LB and R, INS, NS and LP length of 1 cm were placed in culture vessel containing callus induction medium (CIM). The CIM (MS) supplemented with 2.5 mg/l 2,4-D, 1.5 mg/l BAP, 400 mg/l casein hydrolysate, 300 mg/l L-proline and 3% sucrose. Inoculated culture vessels were sealed with paraflim and divided into two groups. The first group was kept under cool-white fluorescent lamps (3000 lux) using 16 hrs photoperiod for white light and the second one at complete dark condition for callus induction (CI). For both groups, other culture conditions were same including temperature (25 ± 2ºC). The days of first callus initiation were considered when explants begun to initiate callus. The age of calli was recorded from callus initiation for each explant.

Two weeks old calli developed from different explants under light and dark condition were placed on CIM separately for further proliferation. In this case, approximately uniform size of calli weighting about 200 mg was considered as initial fresh weight (FWi) and cultured in a sterile glass vessel singly in CIM medium. Six weeks old calli were collected and rinsed with sterile distilled water for 4 - 5 times. Excess surface water of calli was soaked by blotting paper and final fresh weight (FWf) was recorded. Relative growth rate (RGR) of callus was determined using following standard formula by Smith and McComb (1981).

$$RGR = \frac{FWf - FWi}{FWi}$$

For plant regeneration, four weeks old calli developed under light and dark conditions were transferred to regeneration medium MS + 2.0 mg/l BAP + 0.5 mg/l NAA + 1.0 mg/l kinetin. Regenerated shoots were placed on ½MS medium without any growth regulators for rooting. In all the media, agar was 0.8% and the pH was 5.8.

The average or mean values were computed from ten replications with standard error (±SE). Analysis of variance (ANOVA), t-test and DMRT were done using SPSS 17.0 software.

**Results and Discussion**

Effect of light and dark on callus induction (CI) was tested using five types of explants showed in Figs. 1, 2 and 3. For each explants, t-value showed that the frequencies differed significantly at high probability (Table 1). Among the varieties frequencies of CI ranged between 29.20 and 97.20% in light, and in dark it was 24.40 to 72.80%. By analyzing the variance significant differences at $p \leq 0.05$ found within the varieties and also in the explants under both conditions (Table 3 and 4). The highest difference (range) 72.80 to 97.20% was observed between light and dark conditions for the explants leaf blade of Motihari variety (Table 1).

Under white light, callus initiated earlier than dark condition. Out of five explants, earliest callusing initiated at $5^{th}$ day from NS of Motihari variety in light condition. The highest amount of callus initiation (80.53%) was found between 5 and 11 d from NS of Motihari variety in light, while calli was not initiated from root explants of all tested varieties in dark condition (Table 1). However, it was reported that in both conditions calli were initiated, while different in morphology and physiology in tobacco (Yanjie 2004). We describe the similar findings as the induced calli showed different morphology on colour and growth in different light conditions. In white light the calli was fully green in color with vigorous growth for all the varieties and explants

**Table 1. Effect of light on callus initiation and induction in three tobacco varieties.**

| Variety | Explants | WL/D condition | NIE | Callus initiation | | | Callus induction | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | A (d) | B (%) | C (%) | TCI | FCI (% ± SE) | t-value | Sig. |
| Motihari | R | WL | 250 | 9.14 | 17.12 | 29.28 | 116 | 46.40 ± 2.17 | 4.86 | 0.000 |
| | | D | 250 | 12.07 | 0.00 | 32.80 | 82 | 32.80 ± 1.77 | | |
| | INS | WL | 200 | 7.25 | 48.42 | 33.08 | 163 | 81.50 ± 1.98 | 6.02 | 0.000 |
| | | D | 200 | 8.41 | 33.51 | 29.99 | 127 | 63.50 ± 2.24 | | |
| | NS | WL | 150 | 5.03 | 80.53 | 10.14 | 136 | 90.67 ± 2.67 | 5.86 | 0.000 |
| | | D | 150 | 5.82 | 45.31 | 23.36 | 103 | 68.67 ± 2.64 | | |
| | LP | WL | 250 | 8.15 | 33.86 | 41.74 | 189 | 75.60 ± 1.11 | 9.52 | 0.000 |
| | | D | 250 | 10.22 | 18.24 | 38.96 | 143 | 57.20 ± 1.58 | | |
| | LB | WL | 250 | 6.21 | 38.65 | 58.55 | 243 | 97.20 ± 1.69 | 10.28 | 0.000 |
| | | D | 250 | 7.55 | 32.11 | 40.69 | 182 | 72.80 ± 1.67 | | |
| Jati | R | WL | 250 | 9.12 | 12.44 | 26.76 | 98 | 39.20 ± 1.31 | 5.67 | 0.000 |
| | | D | 250 | 12.31 | 0.00 | 27.20 | 68 | 27.20 ± 1.67 | | |
| | INS | WL | 200 | 6.75 | 28.43 | 45.57 | 148 | 74.00 ± 2.08 | 3.80 | 0.001 |
| | | D | 200 | 8.57 | 23.52 | 34.98 | 117 | 58.50 ± 5.53 | | |
| | NS | WL | 150 | 5.69 | 67.31 | 18.02 | 128 | 85.33 ± 2.18 | 6.50 | 0.000 |
| | | D | 150 | 6.11 | 42.30 | 23.03 | 98 | 65.33 ± 2.18 | | |
| | LP | WL | 250 | 8.86 | 22.84 | 47.96 | 177 | 70.80 ± 1.98 | 6.27 | 0.000 |
| | | D | 250 | 10.84 | 17.26 | 37.14 | 136 | 54.40 ± 1.71 | | |
| | LB | WL | 250 | 5.76 | 53.25 | 36.35 | 224 | 89.60 ± 2.81 | 5.24 | 0.000 |
| | | D | 250 | 7.41 | 28.63 | 42.17 | 177 | 70.80 ± 2.24 | | |
| Virginia | R | WL | 250 | 9.85 | 7.81 | 21.39 | 73 | 29.20 ± 1.04 | 2.76 | 0.013 |
| | | D | 250 | 12.53 | 0.00 | 24.40 | 61 | 24.40 ± 1.39 | | |
| | INS | WL | 200 | 7.25 | 25.64 | 39.86 | 131 | 65.50 ± 2.41 | 4.23 | 0.001 |
| | | D | 200 | 9.07 | 17.33 | 36.17 | 107 | 53.50 ± 1.50 | | |
| | NS | WL | 150 | 6.54 | 53.81 | 16.86 | 106 | 70.67 ± 2.04 | 3.64 | 0.002 |
| | | D | 150 | 7.21 | 40.72 | 19.95 | 91 | 60.67 ± 1.85 | | |
| | LP | WL | 250 | 8.14 | 16.54 | 34.66 | 128 | 51.20 ± 1.31 | 4.28 | 0.000 |
| | | D | 250 | 10.23 | 12.11 | 30.69 | 107 | 42.80 ± 1.47 | | |
| | LB | WL | 250 | 6.81 | 48.55 | 23.45 | 180 | 72.00 ± 1.03 | 10.62 | 0.000 |
| | | D | 250 | 8.24 | 23.74 | 27.46 | 128 | 51.20 ± 1.67 | | |

WL: White light, D: Dark, NIE: Number of inoculated explants, A: Days of first callus initiation, B: Frequency of callus initiation within 5 - 11 d, C: Frequency of callus initiation within 12 - 18 d, TCI: Total callus induction, FCI: Frequency of callus induction, R: Root, INS: Internodal segment, NS: Nodal segment, LP: Leaf petiole, LB: Leaf blade, Sig.: Significance.

used except root. The calli induced from root were not enough green like other explants and its growth was also poor. It showed the minimum relative growth rate (RGR) among the explants tested. Values of the RGR ranged between 0.91 and 4.58 and RGRs were favoured by white light than dark condition for each explant (Fig. 4). In respect of growth rate significant differences were observed in both conditions among the varieties and explants (Table 3).

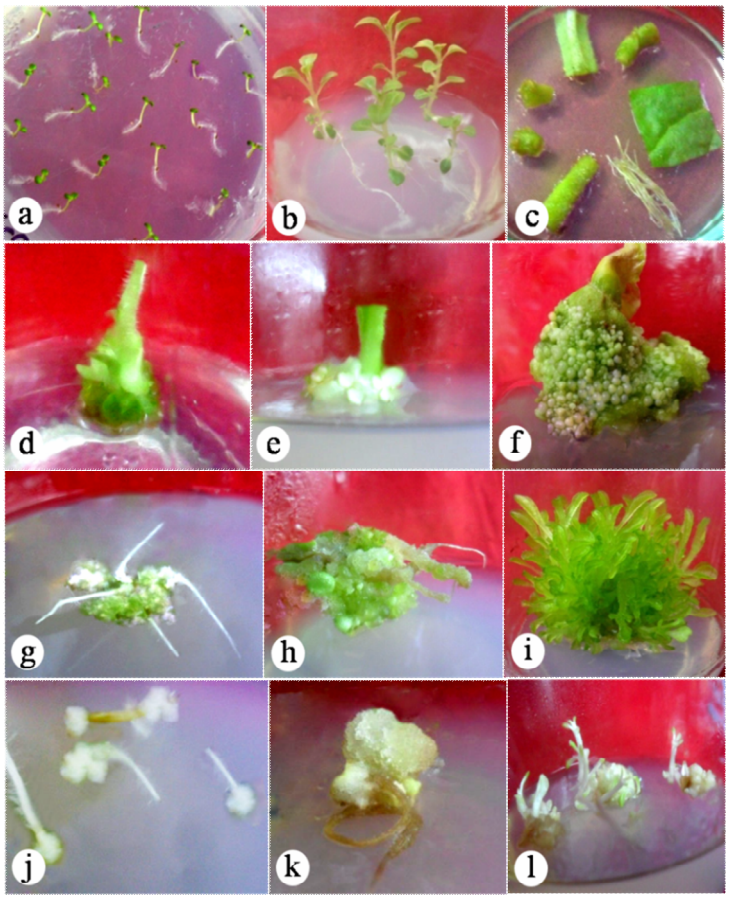


Fig. 1. *In vitro* seed germination, CI and plant regeneration from different explants of Motihari (a - l). (a) Germinated seeds. (b) 15 d old seedlings. (c) Inoculated explants. (d) CI from INS in light. (e) CI from LP in dark. (f) CI from LB in light. (g - i) CI and plant regeneration from root in light. (j - l) CI and plant regeneration from root in dark.

Soheilikhah *et al.* (2013) measured the relative growth rates (RGRs) as 1.3, 4.1, 2.6, 0.9 and 1.2 for five safflower (*Carthamus tinctorius* L.) genotypes *viz.* LRV-51-51, Lesaf, Gila, Kino-76 and Isfahan respectively. In another report the relative growth rate appeared to be remarkably influenced by genotype (Balian *et al.* 2014). Siddique *et al.* (2014) described the effect of genotype to RGR in Bangladeshi *indica* rice varieties; and we observed the genotypic variations. Under white light, the cells of induced calli carried photosynthetic pigments which made them autotrophic in nature. Therefore, the calli might produce carbohydrates and other related, necessary metabolites in their cells and grow vigorously in light than dark condition. Kami *et al.* (2010) showed that light

sensors mediate numerous adaptive responses and developmental transitions. In *Brassica* species effect of light was tested and noticed that callus induction and its growth are stimulated by light (Afshari *et al.* 2011). Paul *et al.* (2013) mentioned that the significant and positive correlation with the yield of plant in both genotypic and phenotypic levels of elephant Foot Yam. We noticed that the light condition would be better than dark if the genotype shows suitability.

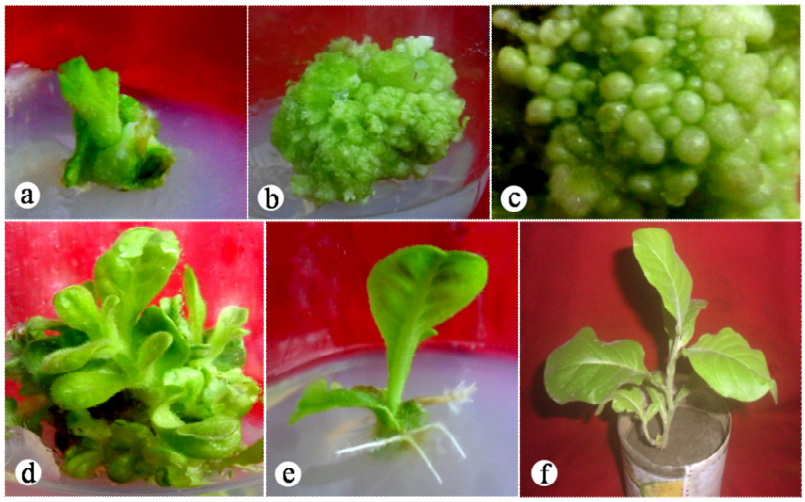


Fig. 2. Callus induction and plant regeneration from the calli induced in white light condition. (a) Callus initiation in nodal segment. (b) Induced callus age of 28 d. (c) Somatic embryogenic in the calli. (d) Regenerated shoots. (e) Well rooted plants. (f) Survived plant in pot culture.

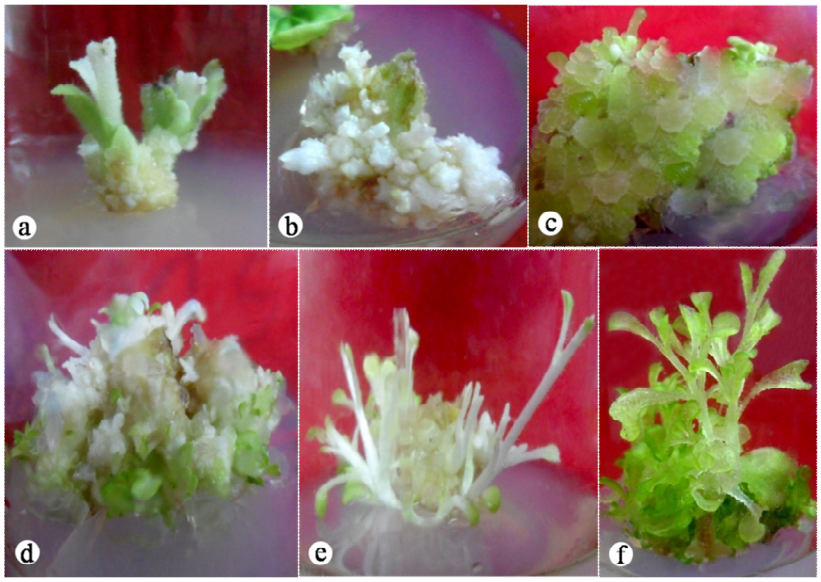


Fig. 3. Callus induction and plant regeneration from the calli induced in dark condition. (a) Callus initiation in nodal segment. (b) Induced callus age of 28 d. (c) Green spots on embryogenic calli. (d) Shoot regeneration. (e) Elongation of shoot. (f) Plantlets developed from the callus produced in absence of light.

**Table 2. Effect of light and dark on plant regeneration in three tobacco varieties.**

| Variety | Explants | WL/D | NIC | Plant regeneration | | | | No. of shoot per callus | | | SRFC | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | NPR | (% ± SE) | t | Sig. | Mean ± SE | t | Sig. | (% ± SE) | t | Sig. |
| Motihari | R | WL | 110 | 32 | 29.09±1.82 | 3.58 | 0.002 | 5.37±0.12 | 15.23 | 0.000 | 61.43±3.72 | 3.21 | 0.005 |
| | | D | 110 | 23 | 20.91±1.39 | | | 3.26±0.08 | | | 46.00±3.06 | | |
| | INS | WL | 110 | 86 | 78.18±2.01 | 14.81 | 0.000 | 8.98±0.16 | 30.07 | 0.000 | 98.57±1.43 | 3.83 | 0.001 |
| | | D | 110 | 45 | 40.91±1.52 | | | 3.62±0.08 | | | 72.00±6.80 | | |
| | NS | WL | 90 | 86 | 95.56±2.46 | 23.49 | 0.000 | 8.59±0.19 | 18.08 | 0.000 | 95.71±2.18 | 3.31 | 0.004 |
| | | D | 90 | 29 | 32.22±1.11 | | | 4.70±0.12 | | | 70.00±7.45 | | |
| | LP | WL | 80 | 63 | 78.75±2.67 | 14.51 | 0.000 | 8.23±0.11 | 26.91 | 0.000 | 94.29±2.33 | 3.69 | 0.002 |
| | | D | 80 | 24 | 30.00±2.04 | | | 4.07±0.08 | | | 70.00±6.15 | | |
| | LB | WL | 120 | 110 | 91.67±3.29 | 14.34 | 0.000 | 10.24±0.20 | 21.15 | 0.000 | 97.14±1.90 | 3.68 | 0.002 |
| | | D | 120 | 45 | 37.50±1.86 | | | 5.15±0.14 | | | 74.00±6.00 | | |
| Jati | R | WL | 110 | 31 | 28.18±0.91 | 5.40 | 0.000 | 5.96±0.13 | 11.29 | 0.000 | 54.29±3.56 | 2.77 | 0.013 |
| | | D | 110 | 22 | 20.00±1.21 | | | 3.33±0.11 | | | 38.00±4.67 | | |
| | INS | WL | 110 | 82 | 74.55±1.21 | 16.84 | 0.000 | 7.10±0.10 | 22.56 | 0.000 | 95.71±2.18 | 4.65 | 0.000 |
| | | D | 110 | 40 | 36.36±1.92 | | | 2.73±0.13 | | | 66.00±6.00 | | |
| | NS | WL | 90 | 73 | 81.11±2.89 | 13.92 | 0.000 | 7.59±0.19 | 16.36 | 0.000 | 92.86±3.19 | 3.31 | 0.004 |
| | | D | 90 | 29 | 32.22±1.99 | | | 4.17±0.08 | | | 68.00±6.80 | | |
| | LP | WL | 80 | 57 | 71.25±2.67 | 11.54 | 0.000 | 7.31±0.14 | 14.11 | 0.000 | 91.43±4.86 | 3.57 | 0.002 |
| | | D | 80 | 26 | 32.50±2.04 | | | 4.28±0.11 | | | 66.00±5.21 | | |
| | LB | WL | 120 | 95 | 79.17±2.56 | 14.51 | 0.000 | 9.17±0.26 | 11.43 | 0.000 | 97.14±2.86 | 4.32 | 0.000 |
| | | D | 120 | 39 | 32.50±1.94 | | | 5.32±0.15 | | | 68.00±6.11 | | |
| Virginia | R | WL | 110 | 29 | 26.36±1.63 | 2.06 | 0.054 | 4.98±0.15 | 7.759 | 0.000 | 48.57±3.16 | 2.39 | 0.028 |
| | | D | 110 | 24 | 21.82±1.48 | | | 3.37±0.10 | | | 34.00±5.21 | | |
| | INS | WL | 110 | 67 | 60.91±2.37 | 8.32 | 0.000 | 6.62±0.16 | 10.72 | 0.000 | 88.57±3.56 | 5.18 | 0.000 |
| | | D | 110 | 36 | 32.73±2.42 | | | 4.13±0.11 | | | 60.00±4.22 | | |
| | NS | WL | 90 | 61 | 67.78±2.59 | 12.29 | 0.000 | 6.86±0.19 | 16.76 | 0.000 | 90.00±3.72 | 4.38 | 0.000 |
| | | D | 90 | 26 | 28.89±1.81 | | | 2.98±0.15 | | | 58.00±6.29 | | |
| | LP | WL | 80 | 51 | 63.75±2.24 | 11.88 | 0.000 | 5.85±0.19 | 6.04 | 0.000 | 87.14±3.96 | 3.14 | 0.006 |
| | | D | 80 | 23 | 28.75±1.91 | | | 4.33±0.11 | | | 62.00±6.96 | | |
| | LB | WL | 120 | 81 | 67.50±2.31 | 14.23 | 0.000 | 6.97±0.23 | 11.82 | 0.000 | 91.43±3.16 | 3.78 | 0.001 |
| | | D | 120 | 35 | 29.17±1.39 | | | 4.00±0.09 | | | 64.00±6.53 | | |

WL: White light, D: dark, NIC: Number of inoculated callus, NPR: Number of plant regeneration, SRFC: Survival rate in field culture, t = t-value and Sig.: Significance.

The calli which developed from light regenerated significantly higher than the dark condition (Tables 2 and 3). The frequency of plant regeneration ranged between 26.26 and 95.56% in light

condition, while 20.0 to 40.91% in dark. Among the explants, the highest plant regeneration was found from the calli of NS that induced in light for Motihari variety and the lowest from root of Jati variety. The highest number of shoots per callus was recorded 10.24 from LB of Motihai in light and the lowest 2.73 from INS of Jati variety in dark condition. The ranges of survival rate of the regenerated plants were found 48.57 to 98.57% and 38.0 to 74.0% in light and dark condition respectively.

**Table 3. Analysis of variance subjected to callus initiation, callus induction, RGRs, plant regeneration, shoot numbers and survival rate of plants in field culture.**

| Source of variation | Df | Mean sum of square (data source) | | | |
|---|---|---|---|---|---|
| | | CI (Table 1) | RGR (Fig. 4) | PR (Table 2) | NSPC (Table 2) |
| Variety | 2 | 715.18*** | 2.64*** | 288.38*** | 3.69** |
| Explants | 4 | 1761.10*** | 4.27*** | 1105.38*** | 4.50*** |
| Light condition | 1 | 1842.40*** | 9.54*** | 9624.12*** | 84.61*** |
| Error | 22 | 15.97 | 0.120 | 101.955 | 0.634 |

CI = Callus induction, RGR = Relative growth rate, PR = Plant regeneration, NSPC = Number of shoots per callus, DF = Degrees of freedom, ** = Significant at $p \leq 0.01$, *** = Significant at $p \leq 0.001$

**Table 4. Efficiency of varieties explants and light condition on different parameters.**

| Variable | | Mean | | | |
|---|---|---|---|---|---|
| | | Callus induction | Plant regeneration | No. of shoot/callus | SRFC |
| Variety | Motihari | 68.63a | 53.48a | 5.01c | 77.91a |
| | Jati | 63.52b | 48.78ab | 5.70ab | 73.74b |
| | Virginia | 52.11c | 42.77b | 6.22a | 68.37c |
| Explants | R | 33.20d | 24.39b | 4.38c | 47.05b |
| | INS | 66.08b | 53.94a | 5.53b | 80.14a |
| | NS | 73.56a | 56.30a | 5.82b | 79.10a |
| | LP | 58.66c | 50.83a | 5.68b | 78.48a |
| | LB | 75.60a | 56.25a | 6.81a | 81.95a |
| Light condition | White light | 69.26 | 66.25 | 7.32 | 85.62 |
| | Dark | 53.59 | 30.43 | 3.96 | 61.07 |

SRFC: Survival rate in field culture, within a variable in each column different letter(s) indicate significant difference according to DMRT at $p \leq 0.05$.

Qualitative features of regenerated shoots were observed visually, and remarkable differences found between light and dark conditions. The shoots developed from the calli that received light were very healthy, deep green in colour with vigorous growth (Fig. 2). On the contrary, very weak shoots with poor growth were produced from the calli induced in dark also showed whitish or

mosaic colour (Fig. 3). The plants developed from weak shoots of dark calli could not be able to survive enough in field condition. Previously it was reported that no significant difference between the treatments darkness and lightness on shoot regeneration in tobacco (Yanjie 2004). Our findings argued with the previous report and noticed that light affected callusing, callus

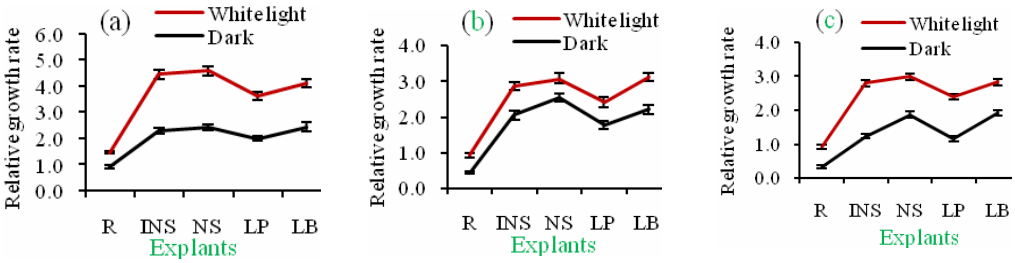


Fig. 4. Relative growth rate of the calli induced in white light and dark condition for the varieties. (a) Motihari, (b) Jati and (c) Virginia.

growth and development of shoot positively. The calli induced in light condition developed shoot with vigorous growth. However, Güler (2009) mentioned that chlorophyll affected yield and related parameters in potato (*Solanum tuberosum* L.). The abilities of photosynthesis increased plant morphological features gradually in *Ginkgo* (Yang and Chen 2014). Present investigation agreed with their reports and claimed that the calli developed in light condition might carry photosynthetic pigments that influenced growth and development of calli. Hence, it could be concluded that for efficient callus induction and plant regeneration calli would be developed in light condition rather than dark in tobacco.

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