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Role of phytohormone on *in vitro* regeneration of tea [*Camellia sinensis* (L.) O. Kuntze]

Mokaram Hanifa Koly[1], Md. Ekramul Hoque[2], Khadiza Khatun[3], Kazi Meftahul Jannat[1] and Md. Rafiqul Islam[4]*

[1]MS student, Department of Biotechnology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh.
[2]Professor, Department of Biotechnology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh.
[3]Professor, Department of Biotechnology, Patuakhali Science and Technology University, Patuakhali, Bangladesh.
[4]Associate Professor, Department of Biotechnology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh.

*Corresponding author. Email: rafiqul@sau.edu.bd; Tel: +880 2-44814048/+8801912939598.

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ABSTRACT: Phytohormones are small molecules present in very low concentrations in plants and provide a promising strategy to improve mass culture biotechnology due to their intrinsic role in growth, development, and survival. This study is aimed at evaluating the effect of different plant growth regulators on *in vitro* callus induction and shoot regeneration in tea. The experiment was conducted at Complete Randomize Design (CRD) with three replications. Callus induction and shoot regeneration was done by using Murashige & Skoog (MS) media supplemented with various concentrations and combinations of Naphthalene Acetic Acid (NAA), Benzyl Adenine (BA), and 2,4-Dichlorophenoxyacetic Acid (2,4-D). Shoot tips and nodal segments were used as explant. The highest percentage (86.51%) of callus induction was observed in the combined treatment of 2.00 milligram per litre (mg/L) BA + 1.50 mg/L 2, 4-D in 2.12 weeks. The same treatment produced the highest weight of calli, which was 0.18, 0.56, and 1.11 grams (g) for 6, 10, and 14 weeks after inoculation (WAI), respectively. The highest percentage (84.39%) of shoot induction was recorded with 2.00 mg/L BA + 2.00 mg/L NAA in 32.43 days. The maximum number (1.42, 2.46, and 4.49) and the highest length of the shoot (1.41, 3.28, and 4.61 cm) at 60, 90, and 120 DAI (days after inoculation) were also recorded from the same treatment. No callus or shoot induction was recorded in the controlled treatment. The protocol developed from the present study might be useful for the large-scale production of healthy, disease-free planting material for tea.

Keywords: Callus induction, MS media, phytohormone, shoot regeneration, tea.

Abbreviations: MS Media = Murashige & Skoog media; **BA=** Benzyl Adenine; **BAP =** 6-Benzylaminopurine; **2,4-D =** 2,4-Dichlorophenoxyacetic Acid**; IAA =** Indole acetic acid; **IBA =** Indole-3-butyric acid; **NAA =** Naphthalene Acetic Acid; **TDZ =** Thidiazuron; **Kn** = Kinetin; **µM =** Micromole; **WAI=** Weeks after inoculation; **DAI =** Days after inoculation.

INTRODUCTION

Tea (*Camellia sinensis* (L.) O. Kuntze) belongs to the genus *Camellia* and is classified in the order Ericales and the family Theaceae. There are more than 200 species in the genus, most of which are native to southern China, the mountains of Tibet, and northern and eastern India. The plant is used to produce the majority of tea beverages. It has several health benefits. Those who frequently consume black or green tea have a lower chance of developing heart disease. Tea may help regulate blood sugar, lowering the risk of type-2 diabetes and the chance of developing certain cancers. One of the top tea-producing nations in the world is Bangladesh. There are

167 commercial tea farms and tea gardens in Bangladesh. Bangladesh produced 96.50 million kilograms (kg) of tea in 2021, and in the fiscal year 2021–2022, it earned 1.956 million USD ($) by exporting 0.639 million kg of tea worldwide (Bangladesh Tea Board, 2023). Conventional tea breeding and its improvement have some drawbacks due to the following factors: a long regeneration cycle, perennial nature, self-incompatibility, high inbreeding depression, absence of appropriate selection criteria, lengthy seed maturation, lack of availability of distinct genotypes of various biotic and abiotic stresses, and a low success rate of hand pollination. Even vegetative propagation has some limitations, like season dependence, poor rooting, and a slow multiplication rate of the cuttings. Winter dormancy causes the unavailableness of planting materials (Mondal *et al*., 2005). Also cutting materials gradually lose their vitality, and if the mother plant is ill, the cuttings could spread the disease as well. For all this incompetence, a huge number of mother plants are required for vegetative propagation, which is neither economically beneficial nor possible every year. In these conditions, micropropagation through tissue culture is necessary for the quick and widespread regeneration of clones. Various techniques for tissue culture of tea have been successfully tested in a number of studies in the past (Agarwal *et al*., 1992; Iddagoda et al., 1988; Arulpragasam and Latiff, 1986; Sandal *et al*., 2001). Very few works are found on tea tissue culture through direct regeneration in Bangladesh. These are not adequate, and additional studies in this field using various approaches and hormone use in tissue culture techniques are required. Considering the above limitations, the present experiment was carried out for direct and indirect regeneration in tea.

MATERIALS AND METHODS

The current study was conducted in the Biotechnology Laboratory of the Sher-e-Bangla Agricultural University from January 2020 to September 2021. The experimental materials were collected from the Regional Office of the Bangladesh Tea Research Institute at Panchagar. The shoot tip and nodal segment were used as explants. The explants were surface disinfected by dipping in 70% ethanol for one minute, then submerged in 0.2% $HgCl_2$ along with 3 to 4 drops of Tween-20 for 4 to 5 minutes and washed three times with sterile distilled water. The sterilized explants were finally cut into 0.20 to 0.50 cm size, placed on agar solidified MS medium (Murashige and Skoog, 1962) supplemented with different concentrations and combinations of PGRs and incubated at 21±2°C where 14 hours of photoperiod, and 3000–3500 lux of light intensity (25W white bulbs) with 70% relative humidity (RH) were maintained. Subculturing was done at regular intervals. Throughout the entire cultural period, observations were conducted regarding the development patterns of calli and shoots. Each treatment consisted of three replicates. Data were collected on the percentage of callus induction, the weight of the callus, the days and the percentage of shoot induction, the number and length of the shoot, and the number of leaves per shoot. In the case of callus proliferation, data were collected at 6, 10, and 14 WAI of culture. Shoot regeneration data was recorded up to 60, 90, and 120 days after inoculation of the explant. Data were statistically analyzed for the analysis of variance (ANOVA) and least significant difference (LSD) at the 5% significance level, and a comparison was made between treatment means.

RESULTS AND DISCUSSION

Callus induction

Statistical analysis for different concentrations of 2,4-D; 2.00 mg/L BA showed significant differences from each other in the case of weeks to callus induction. Initiation of the callus needed a minimum of 2.12 weeks for treatment 2 (2.00 mg/L BA + 1.50 mg/L 2,4-D) and a maximum of 3.41 weeks for treatment 1 (2.00 mg/L BA + 1.00 mg/L 2,4-D). There was no response found in the control treatment for the days of callus initiation (Figure 1). According to Sarwar (1985), the initiation of a callus was found after 42 days of incubation in MS (0.7% agar solidified) medium supplemented with 2% sucrose and 2,4-D+NAA or BA+ Kn.

Percentage of callus induction

Regarding the percentage of callus induction, a significant difference between various doses of 2, 4-D with 2.00 mg/L BA was observed. The rate of callus induction varied among the treatments. The treatment 2 (2.00 mg/L BA + 1.50 mg/L 2, 4-D) produced the highest percentage (86.51%) and the treatment 4 (2.00 mg/L BA + 2.50 mg/L 2, 4-D) showed the lowest percentage (54.14%). The control treatment did not elicit any response (Table 1). According to Seran *et al*. (2007), after 30 minutes of soaking in 60% and 75% solutions of Clorox™, respectively, 50% and 58% aseptic callus cultures were produced from tea leaves.

Fresh weight of callus

It was observed that treatment 2 (2.00 mg/L BA + 1.50 mg/L 2, 4-D) and treatment 3 (2.00 mg/L BA + 2.00 mg/L 2, 4-D) had statistically similar results (0.18 and 0.16 g respectively), and they were significantly different from other treatments in respect of callus weight at 6 WAI. However, at 10 and 14 WAI, the largest callus weights

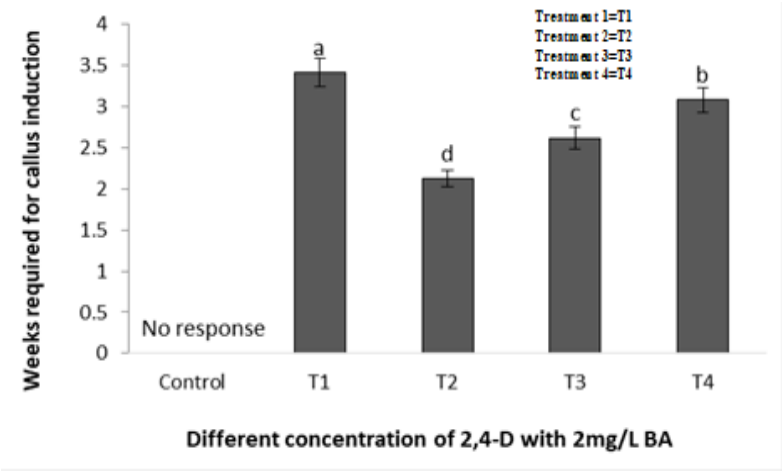

Figure 1. Weeks to callus induction in Tea with different concentration of 2, 4-D with 2.00 mg/L BA.

Table 1. Combined effect of BA and 2, 4-D on percentage of callus induction in tea.

Treatment (BA+ 2,4-D) mg/L	Percentage of callus
Controlled	-
T1 (2.00 + 1.00)	62.48 c
T2 (2.00 + 1.50)	86.51 a
T3 (2.00 + 2.00)	73.48 b
T4 (2.00 + 2.50)	54.14 d
CV (%)	0.43
LSD $_{(0.05)}$	0.565

Figures in a column followed by different letter (s) differ significantly whereas figures having common letter(s) do not differ significantly from each other. CV= Coefficient of variation, LSD $_{(0.05)}$ = Least significant difference.

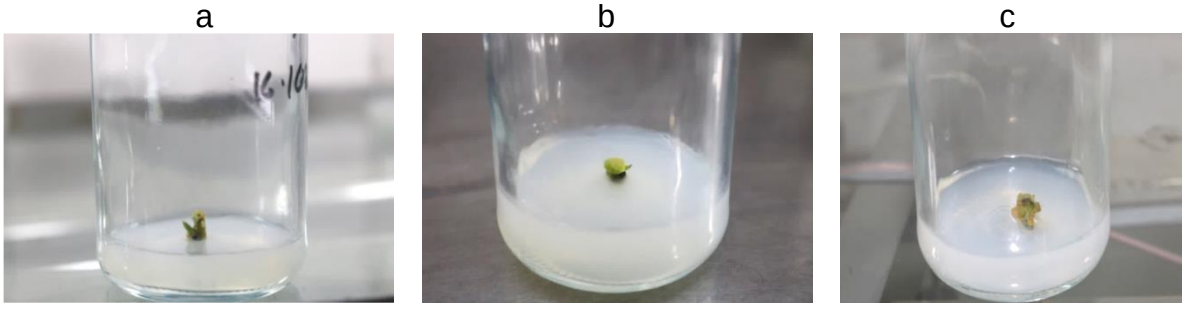

Figure 2. (a). Callus at 6 WAI in the treatment 2.00 mg/L BA + 1.50 mg/L 2, 4-D, **(b).** Callus at 10 WAI in the treatment 2.00 mg/L BA + 1.50 mg/L 2, 4-D, **(c).** Callus at 14 WAI in the treatment 2.00 mg/L BA + 1.50 mg/L 2, 4-D.

(0.56 g and 1.11 g, respectively) were produced by treatment 2 (2.00 mg/L BA + 1.50 mg/L 2,4-D), and in both cases, they exhibited a significant difference from other treatments (Figure 2). On the contrary, Treatment 1 (2.00 mg/L BA + 1.00 mg/L 2,4-D) produced the lowest weight of callus (0.11 and 0.69 g) at 6 WAI and 14 WAI, respectively. However, treatment 4 (2.00 mg/L BA + 2.50 mg/L 2, 4-D) resulted in the least amount of callus (0.38 g)

Table 2. Effect of BA and 2, 4-D on fresh weight of callus at different week after inoculation (WAI).

Treatment (BA+ 2,4-D) mg/L	Weight of callus (g)		
	6 WAI	10 WAI	14 WAI
Controlled	-	-	-
T1 (2.00 + 1.00) mg/L	0.11 c	0.42 bc	0.69 c
T2 (2.00 + 1.50) mg/L	0.18 a	0.56 a	1.11 a
T3 (2.00 + 2.00) mg/L	0.16 a	0.45 b	0.82 b
T4 (2.00 + 2.50) mg/L	0.14 b	0.38 c	0.72 c
CV (%)	4	4.62	2.77
$LSD_{(0.05)}$	0.0111	0.0393	0.0435

*WAI=Weeks after Inoculation. Figures in a column followed by different letter(s) differ significantly whereas figures having common letter(s) do not differ significantly from each other. CV= Coefficient of variation, $LSD_{(0.05)}$ = Least significant difference.

Table 3. Combined Effect of BA and NAA on days and percentage of shoot induction in Tea.

Treatment (BA + NAA) mg/L	Days of shoot induction	Percentage of shoot induction
(Controlled)	-	-
T1 (2.00 + 1.00)	40.45 b	55.32 d
T2 (2.00 + 2.00)	32.43 d	84.39 a
T3 (2.00 + 3.00)	36.83 c	72.15 b
T4 (2.00 + 4.00)	45.77 a	67.82 c
CV (%)	0.47	0.27
$LSD_{(0.05)}$	0.3467	0.3561

Figures in a column followed by different letter(s) differ significantly whereas figures having common letter(s) do not differ significantly from each other. CV= Coefficient of variation, $LSD_{(0.05)}$ = Least significant difference.

at 10 WAI (Table 2). Though the callus formed and its weight increased, no further shoot or root formation was found in the callus. According to Sandal *et al*. (2005) after four weeks of culture on different 2,4-D doses (2.50, 5.00, 7.50, and 10.00 mg/L), callus was induced, but the callus failed to exhibit any shoot formation at low concentrations of Phytohormone. Organogenesis from calluses has also been reported in several studies. After 8 weeks, shoot initiation was observed when callus tissue was placed in a mixed medium containing 0.50 mg/L of Indole-3-butyric acid (IBA) and 10 mg/L of BA. Roots began to form two weeks after the regenerated shoots were placed in the rooting medium (Kato, 1985).

Shoot induction

The varying concentrations of NAA with 2.00 mg/L BA provide insight into the significant variance in days and shoot induction percentage. The results from each treatment were statistically different from one another. The minimum 32.43 days to shoot induction with the highest percentage of the shoot (84.39%) were noticed in treatment 2 (2.00 mg/L BA + 2.00 mg/L NAA), and the maximum 45.77 days to shoot initiation were recorded in treatment 4 (2.00 mg/L BA + 4.00 mg/L NAA). But the lowest percentage (55.32%) was found at treatment 1 (2.00 mg/L BA + 1.00 mg/L NAA). Nevertheless, the controlled therapy did not affect the induction of shoots (Table 3). After comparing results with other hormones Mondal *et al*. (2004) found that 10 µM NAA + 5 µM TDZ (Thidiazuron) in MS media produced the highest shoot (98%). Additionally, these authors mentioned that, in MS, shooting with various hormones might take place anywhere between 4 and 10 weeks. The highest response was 75% for the shoot tip and 66.67% for the nodal segments in the combined treatment of 3.00 mg/L 6-Benzylaminopurine (BAP) + 0.05 mg/L Indole Acetic Acid (IAA), when the lowest 10% and 8% were found in 3.00 mg/L BAP + 0.10 mg/L Kn for these explants, respectively (Begum *et al*., 2015). Various reports revealed that 30–50 days are required for shoot initiation at different concentrations and combinations of auxin and cytokinin. It is also reported that the success percentage of shoot induction depends on the type of explant, the hormones, and their concentrations.

Table 4. Combined effect of BA and NAA on number of shoots at different days after inoculation (DAI).

Treatment (BA + NAA) mg/L	Number of shoots		
	60 DAI	90 DAI	120 DAI
(Controlled)	-	-	-
T1 (2.00 + 1.00)	0.86 d	1.43 d	2.63 c
T2 (2.00 + 2.00)	1.42 a	2.46 a	4.49 a
T3 (2.00 + 3.00)	1.16 b	1.98 b	3.16 b
T4 (2.00 + 4.00)	1.05 c	1.62 c	2.46 c
CV (%)	3.9	4.19	3.82
$LSD_{(0.05)}$	0.0826	0.1478	0.2294

DAI=Days after inoculation. Figure in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other. CV= Coefficient of variation, $LSD_{(0.05)}$ = Least significant difference.

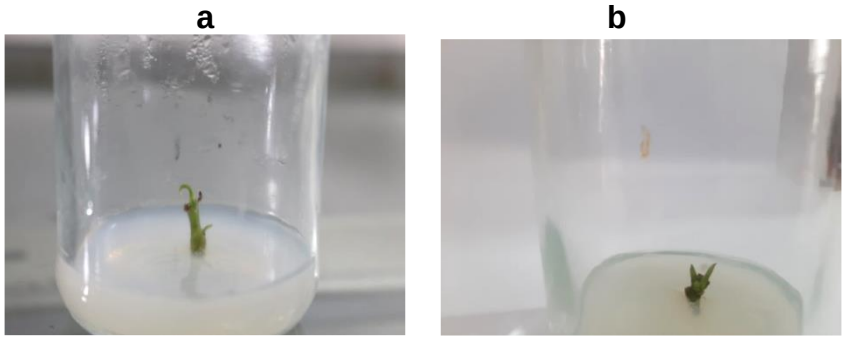

Figure 3. (a). Number of Shoot at 90 DAI in the treatment 2.00 mg/L BA + 2.00 mg/L NAA, (**b).** Number of Shoot at 120 DAI in the treatment 2.00 mg/L BA + 2.00 mg/L NAA.

Number of shoots per explant

All of the treatments are considerably different in respect of shoot number per explant from one another at 60 and 90 DAI (days after inoculation). However, at 120 DAI, treatment 1 (2.00 mg/L BA + 1.00 mg/L NAA) and treatment 4 (2.00 mg/L BA + 4.00 mg/L NAA) showed statistically similar results, while they were significantly different from the other treatments. Treatment 2 (2.00 mg/L BA + 2.00 mg/L NAA) produced the highest number of shoots (1.42, 2.46, and 4.49) at 60, 90, and 120 DAI. On the contrary, treatment 1 (2.00 mg/L BA + 1.00 mg/L NAA) produced the lowest number of shoots (0.86 and 1.43) at the 60 and 90 DAI, respectively. But at 120 DAI, the lowest number of shoots (2.46) had been produced in treatment 4 (2.00 mg/L BA + 4.00 mg/L NAA) (Table 4 and Figure 3). These results showed that there was a significant influence of different concentrations of BA and NAA on the number of shoot regenerations in tea plants. Even after a lengthy amount of time and numerous subcultures, the proliferation of the controlled treatment was not detected. Similar results were found by other authors (Jha and Sen, 1992). They discovered that hormone-free basal media produced no reaction. A medium containing both BA and auxins generated more shoots than a medium simply containing cytokinins (Khan *et al*., 1997). In every nodal segment, after 2 months, 3 to 4 shoot buds were generated without callus formation when the culture medium was supplemented with 8.00 mg/L Kn + 2.00 mg/L IAA (Kumari *et al*., 1984).

Length of shoot

Each treatment displayed significantly different outcomes from one another. The longest shoots (1.41, 3.28, and 4.61 cm) were produced by Treatment 2 (2.00 mg/L BA + 2.00 mg/L NAA) at 60, 90, and 120 DAI, respectively. The least number of shoots (0.80, 1.85, and 2.85 cm) were produced by treatment 3 (2.00 mg/L BA + 3.00 mg/L NAA) at 60, 90, and 120 DAI, but no response observed at the controls (Table 5 and Figure 4). After 120 days, the combined concentration of 2.00 mg/L BAP + 2.00 mg/L NAA produced 3.80-4.00 cm long shoot (Boonerjee *et al*., 2013)

Table 5. Combined effect of BA and NAA on length of shoot at different days after inoculation (DAI).

Treatment (BA + NAA) mg/L	length of shoot (cm)		
	60 DAI	90 DAI	120 DAI
(Controlled)	-	-	-
T1 (2.00 + 1.00)	0.97 c	2.70 b	3.97 b
T2 (2.00 + 2.00)	1.41 a	3.28 a	4.61 a
T3 (2.00 + 3.00)	0.80 d	1.85 d	2.85 d
T4 (2.00 + 4.00)	1.12 b	2.31 c	3.36 c
CV (%)	5.3	1.61	1.53
LSD $_{(0.05)}$	0.1078	0.0768	0.1067

DAI=Days after inoculation. Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other. CV= Coefficient of variation, LSD $_{(0.05)}$ = Least significant difference.

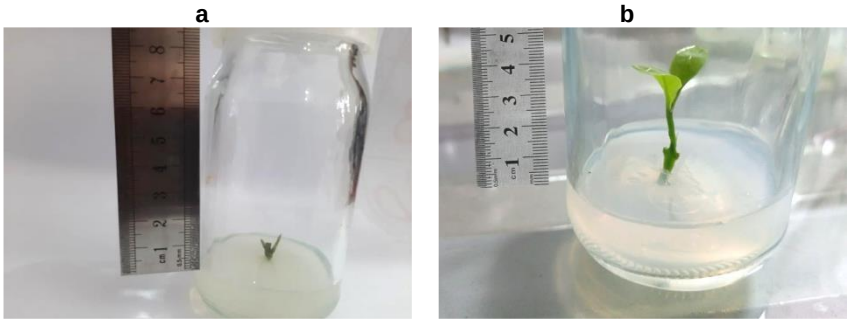

Figure 4. (a). Length of shoot at 60 DAI in the treatment 2.00 mg/L BA + 2.00 mg/L NAA, (**b).** Length of shoot at 120 DAI in the treatment 2.00 mg/L BA + 2.00 mg/L NAA.

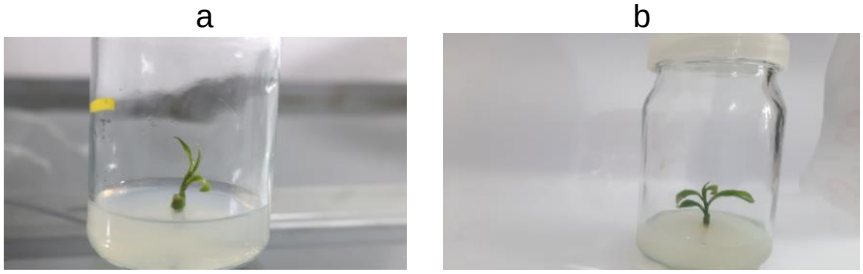

Figure 5. (a). Number of leaves at 90 DAI in the treatment 2.00 mg/L BA and 3.00 mg/L NAA (**b)**. Number of leaves at 120 DAI in the treatment 2.00 mg/L BA and 3.00 mg/L NAA.

which is almost identical to present research. Though their best result was founded in 2.00 mg/L BAP with the combination of 0.10 mg/L IBA, the results were 4.50 cm for the shoot tip and 5.00 cm for the nodal segment.

Number of leaves

Treatment 3 (2.00 mg/L BA + 3.00 mg/L NAA) differed significantly from treatments 1 (2.00 mg/L BA + 1.00 mg/L NAA) and treatment 2 (2.00 mg/L BA + 2.00 mg/L NAA) at 90 DAI, but by 120 DAI for the number of leaves, all treatments were statistically different from one another. The largest number of leaves (3.36 and 5.53) were produced by treatment 3 (2.00 mg/L BA + 3.00 mg/L NAA), whilst the lowest number (2.13 and 3.85) were produced by treatment 1 (2.00 mg/L BA + 1.00 mg/L NAA) at 90 and 120 DAI, respectively. No response was established in the controlled treatment for the number of leaves at various DAI (Figures 5 and 6). After culturing and sub-culturing explants for 4 to 6 weeks, four to six leaves were reported (Prakash *et al*., 1999).

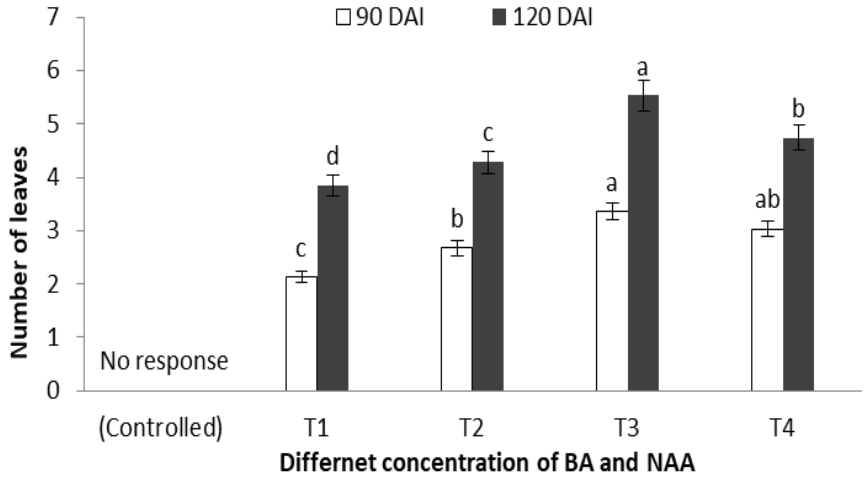

Figure 6. Combined effect of BA and NAA on number of leaves at different DAI.

Conclusion

Both direct and indirect (from callus) regeneration of tea were studied in the present experiment. Although a significant volume of callus was created, further regeneration from callus was not observed, even after extensive sub-culturing and a long period of time. But an adequate number of shoots and leaves were produced through direct regeneration. Treatment 2 (2.00 mg/L BA + 1.50 mg/L 2,4-D) had the highest callus weight at 10 and 14 WAI, while treatment 1 (2.00 mg/L BA + 1.00 mg/L 2, 4-D) had the lowest weight at 6 and 14 WAI. Again, different NAA concentrations with 2.00 mg/L BA had a significant effect on shoot induction, percentage, and days. Treatment 2 (2.00 mg/L BA + 2.00 mg/L NAA) produced the most shoots and the longest shoots, while treatment 3 (2.00 mg/L BA + 3.00 mg/L NAA) produced the highest number of leaves. The controlled therapy had no effect on shoot induction. In conclusion, direct regeneration can be achieved by applying both the auxin and cytokinin groups of hormones.

CONFLICTS OF INTERESTS

The authors declare that there are no conflicts of interest to publish in this journal.

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REFERENCES

Agarwal, B., Singh, U., & Banerjee, M. (1992). In vitro clonal propagation of tea (*Camellia sinensis* (L.) O. Kuntze). *Plant Cell, Tissue and Organ Culture*, *30*(1), 1-5.

Arulpragasam, P. V, & Latiff, R. (1986). Studies on the tissue culture of tea (*Camellia sinensis* (l.) O. Kuntzej. 1. Development of aculture method for the multiplication of shoots. *Sri Lanka Journal of Tea Science, 55*(1), 44-47.

Bangladesh Tea Board. (2023). *Statistical Bulletin of Bangladesh Tea Board for the Month of January, 2023*. Retrieved from http://www.teaboard.gov.bd/site/monthly_report/2e7d9840-d1d4-43c6-b1a4-a4463a4417cb

Begum, A., Ahmad, I., Prodhan, S. H., Azad, A. K., Sikder, M. B. H., & Ara, M. R. (2015). Study on in vitro propagation of tea [Camellia sinensis (L.) O. Kuntze] through different explants. *Journal of Global Biosciences, 4*(7), 2878-2887.

Boonerjee, S., Hoque, M. I., & Sarker, R. H. (2013). Development of in vitro micro propagation system in Tea plant [*Camellia sinensis* (L.) O. Kuntze] using shoot tip and nodal segment explants. *Tea Journal of Bangladesh*, *42*, 21-30.

Iddagoda, N., Kataeva, N. N., & Butenko, R. G. (1988). *In vitro* clonal micropropagation of tea (*Camellia sinensis* L.) 1. Defining the optimum condition for culturing by means of a mathematical design technique. *Indian Journal of Plant Physiolog, 31*, 1-10.

Jha, T., & Sen, S. K. (1992). Micropropagation of an elite Darjeeling tea clone. *Plant Cell Reports*, *11*(2), 101-104.

Kato, M. (1985). Regeneration of plantlets from tea stem callus. *Japanese Journal of Breeding*, *35*(3), 317-322.

Khan, P. S. S. V., Prakash, E., & Rao, K. R. (1997). In vitro micropropagation of an endemic fruit tree *Syzygium alternifolium* (Wight) walp. *Plant Cell Reports*, *16*(5), 325-328.

Kumari, M., Nee, P., & Mitra, G. C. (1984). Regeneration of tea shoots from nodal explants in tissue culture. *Current Science*, *53*(16), 874-876.

Mondal, T. K., Bhattacharya, A., Laxmikumaran, M., & Ahuja, P. S. (2004). Recent advances of tea (*Camellia sinensis*) biotechnology. *Plant Cell, Tissue and Organ Culture*, *76*(3), 195-254.

Mondal, T. K., Parathiraj, S., & Kumar, P. M. (2005). Micrografting: A technique to shorten the hardening time of micropropagated shoots of tea (*Camellia sinensis* (L) O. Kuntze). *Sri Lanka Journal of Tea Science, 70*(1), 5–9.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, *15*(3), 473-497.

Prakash, O., Sood, A., Sharma, M., & Ahuja, P. S. (1999). Grafting micropropagated tea [*Camellia sinensis* (L.) O. Kuntze] shoots on tea seedlings - A new approach to tea propagation. *Plant Cell Reports*, *18*(10), 883-888.

Sandal, I., Bhattacharya, A., & Singh Ahuja, P. (2001). An efficient liquid culture system for tea shoot proliferation. *Plant Cell, Tissue and Organ Culture*, *65*(1), 75-80.

Sandal, I., Kumar, A., Bhattacharya, A., Sharma, M., Shanker, A., & Ahuja, P. S. (2005). Gradual depletion of 2,4-D in the culture medium for indirect shoot regeneration from leaf explants of *Camellia sinensis* (L.) O. Kuntze. *Plant Growth Regulation*, *47*(2), 121-127.

Sarwar, M. (1985). Callus formation from explanted organs of tea (*Camellia sinensis* L.). *Journal of Tea Science, 54*(1), 18-22.

Seran, T. H., Hirimburcgama, K., & Gunasckare, M. T. K. (2007). Establishment of in vitro culture to produce friable callus from leaf of *Camellia sinensis* (L.). *Agrieast*, *6*, 49-58.