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RESEARCH PAPER

In Vitro Regeneration of Strawberry (*Fragaria* × *ananassa* Duch.)

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ABSTRACT

Strawberry (*Fragaria* × *ananassa* Duch.), a popular fruit in Bangladesh, traditionally grows in temperate climates and spreads through runners, which are susceptible to diseases and have lower survival rates. To address this, the current study focuses on mass propagating disease-resistant explants. Using *in vitro* methods, strawberry seedlings are produced that can withstand diseases and thrive in any season, regardless of climate. The healthy, disease free shoot tips were used as explants, sterilized by 0.1% $HgCl_2$ mixed with few drops of Tween-20 and inoculated in MS media supplemented with different combination of the BA (1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) and IBA (0.50, 1.00, 1.50, 2.00 and 2.50 mg/l) either alone or in combination. The highest shoot count (3.60) was seen with 3.0mg/l BA, and the most leaves (9.60) with 2.0 mg/l BA. However, a combination of 2.0 mg/l BA+1.00 mg/l IBA yielded the best shoot numbers, while 2.0 mg/l BA+0.5 mg/l IBA produced the most leaves per explant. The highest number of roots was observed in 2.0 mg/l BA+ 1.5 mg/l IBA and the highest length of root was observed in 2.0 mg/l BA+1.0 mg/l IBA. Single treatments of 2.0 mg/l IBA and 1.50 mg/l IBA respectively produced the highest root counts (4.00) and lengths (3.34). Overall, micro-propagation proved a highly effective and promising method for strawberry proliferation that can be used to enhance breeding programs.

HIGHLIGHTS

- Micro-propagation proved to be an effective and promising method for strawberry proliferation, offering a valuable tool for breeding programs.

Keywords: Strawberry, BA, IBA, Micro-propagation.

Strawberry (*Fragaria* × *ananassa* Duch.) is a perennial, stoloniferous hybrid species of genus Fragaria from the Rosaceae family, widely cherished for its distinctive red color, juicy texture, flavor and rich nutritional profile, including high levels of anthocyanins which contribute to its antioxidant properties (Monzur *et al.* 2013; Giampieri *et al.* 2016; Ulrich *et al.* 2018). These compounds have been noted to aid in reducing blood pressure and alleviating stress (Zahedi *et al.* 2020). Globally, strawberry ranks among the top fruits in terms of consumption and cultivation, with its production spanning 85 countries and covering 389,665 hectares, yielding 9,175,384.43 metric tons annually (FAOSTAT, 2021).

In Bangladesh, the journey of strawberry cultivation began in 1996, and it took over a decade to develop varieties suited to the local climate. Notably, the

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Bangladesh Agriculture Research Institute (BARI) introduced a high-yielding variety named 'BARI Strawberry-1'. Additionally, Rajshahi University and the Modern Horticulture Center in Natore have developed several other varieties tailored for local conditions (Anon., 2016). Currently, approximately 6,500 bighas of land across the country are dedicated to strawberry farming, with Rajshahi district leading in production.

Runners arising from axillary buds on the plant crown are traditionally utilized for the propagation of strawberries. With this method, only a limited number of plantlets can be produced, the plantlets have poor quality, and pathogens are transferred from the stock plant, which limits agricultural yield. This is particularly crucial for viral diseases, as viruses are transported via vascular bundles (Quiroz *et al.* 2017) and vulnerability to diseases like Strawberry mottle virus (SMoV) and Strawberry mild yellow edge virus (SMYEV), which pose significant threats to production (Martin and Tzanetakis, 2013). In contrast, tissue culture methods offer a more disease free and high yielding alternative, making it preferable for commercial cultivation. The micro-propagation technique allows for the generation of thousands of explants from a small tissue sample in any season, overcoming climatic limitations. The success of this method is significantly influenced by the correct combination and concentration of growth regulators, vitamins, amino acids, and the physical state of the growth media (Kadhimi *et al.* 2014). Therefore, our study aims to evaluate the combined effects of auxins and cytokinins on *in vitro* regeneration of strawberry, identify the optimal hormonal treatment and establish a feasible *in vitro* regeneration protocol for strawberry cultivation.

MATERIALS AND METHODS

Healthy, disease free runner tips of 0.50 cm length were used as explants for *in vitro* regeneration of strawberry. The trimmed strawberry shoot tips were then washed thoroughly under running tap water and sterilized with distilled water for several times. Later, they were transferred to a laminar airflow cabinet, treated with 70% ethanol for 1-2 minutes and rinsed with sterilized distilled water for 3-4 times. Then, the explants were immersed in 0.1% $HgCl_2$ within a beaker and 3-4 drops of Tween-20 were added for about 4-5 minutes with constant shaking in clockwise and anticlockwise direction. Afterwards, the strawberry explants were washed 3-4 times with autoclaved distilled water, trimmed with sterile scalpel blade and after cutting explants into suitable size (0.25-0.50 cm), explants were transferred to culture bottles containing MS medium with plant growth regulator. For incubation, the cultures were kept in culture racks maintained at 21±1 °C with light intensity varying from 4000–5000 lux (23 W white bulbs). The photoperiod was generally 14 hours' light and 10 hours dark having 70% relative humidity (RH).

RESULTS AND DISCUSSION

Micropropagation is an *in vitro* clonal propagation method using small pieces of plant tissues taken from a mother plant is called explant, and grows under laboratory conditions to produce huge plants within a short period of time. Micropropagation is the most popular method for the commercial-scale production of strawberry plantlets (Boxus, 1989); A significant number of runners and suitable planting stock are necessary for the successful planting of strawberries. There are numerous combinations of plant growth regulators that can be used to propagate strawberries, either by runner/nodal segments (Anuradha *et al.* 2016; Jhajhra *et al.* 2018) or by runner tips *in vitro* micropropagation. There were significant differences on different concentrations of BA and IBA on the number of shoots per explant and shoot length, root numbers and root length.

Effect of BA on *in vitro* shoot regeneration potentiality

The treatment BA 2.0 mg/l gave the highest number of shoots at 28 DAI (Days after induction). The highest length of shoot (3.12 cm) at 28 DAI was found at 3.0 mg/l BA and second highest length (2.90 cm) with 1.0 mg/l BA at 28 DAI (Table 1) was observed. The control treatment gave the lowest length (2.04 cm) of shoot which was statistically similar with 2.0 mg/l (2.14 cm) at 28 DAI (Table 1). Naing *et al.* (2019) reported that the culture of meristems in 0.5 mg L^{-1} Kn is suitable for the efficient propagation of different strawberry cultivars which was similar with the findings of Singh *et al.* (2022). On the other hand, Lawand *et al.* (2022) studied that BA at a concentration of 1.0 mg/l had the highest mean number of shoot/explant.

Previous studies reported that the application of low concentrations of cytokinins (Biswas *et al.* 2009; Keiko *et al.* 2003), a reduced number of subcultures during the proliferation stage (da Fonseca *et al.* 2013), and the choice of genotypes (Kaushal *et al.* 2004) are critical factors that should be considered for obtaining true-to-type plants.

Table 1: Effect of different concentration of BA on number of shoots & number of leaves at different DAI

BA(mg/l)	Number of Shoots	Length of Shoot
0.0	1.60b	2.04e
1.0	2.60b	2.90b
2.0	3.60a	2.58c
3.0	2.20b	3.12a
4.0	1.80b	2.36d
5.0	2.20b	2.14e
CV (%)	37.14	5.32
$LSD_{(0.05)}$	1.1796	0.175

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

Effect of IBA on *in vitro* root regeneration potentiality

The treatment IBA 2.0 mg/l gave the highest number of root (4.0) and the highest length of root (2.22cm) was found in 1.50 mg/l IBA at 28 DAI (Table 2). According to Dhukate *et al.* (2021) *in vitro* raised shoots were treated with 500 mg L^{-1} indole-3-butyric acid (IBA) solution for 30 s before inoculating in rooting media and then were cultured in 1 mg/ L IBA, 0.1% activated charcoal and 6% table sugar.

Table 2: Effect of different concentration of IBA on number of root and root length

IBA (mg/l)	Number of Roots	Length of root (cm)
0	2.00d	1.20e
0.5	3.60bc	2.72b
1.0	3.80ab	2.84b
1.5	2.80cd	3.34a
2.0	4.00a	2.22c
2.5	2.40d	1.62d
CV (%)	21.21	6.26
LSD (0.05)	0.923	0.1899

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

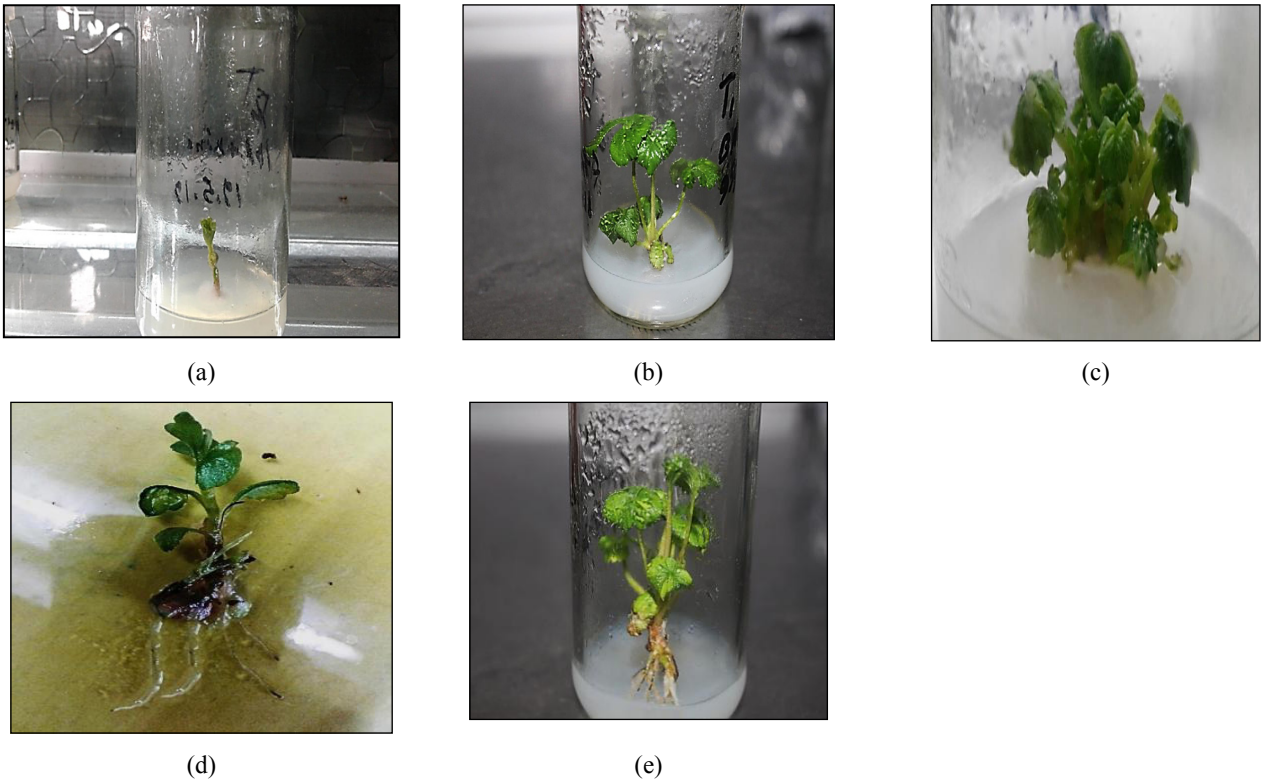
Combined effect of BA and IBA on *in vitro* plantlet regeneration

The treatment BA 2.0 mg/l +IBA 1.0 mg/l gave the highest number of shoot (4.40) at 28 DAI. The maximum length of shoot (3.66 cm) at 28 DAI was noticed from the BA 2.0 mg/l + IBA 2.0 mg/l which were statistically different from rest of others. It was the minimum (1.08 cm) at 28 DAI in control (Table 3). Labade *et al.* (2016) found that MS medium

Table 3: Combined effect of BA and IBA on *in vitro* regeneration of Strawberry

Treatment (mg/L)	Number of Shoots	Shoot length	Root Number	Root length
BA 1.0 + IBA 0.5	2.62b-e	2.56c	4.00ab	3.34d
BA 1.0 + IBA 1.0	3.20a-c	2.48cd	3.4b-d	3.26de
BA 1.0 + IBA 1.5	2.80a-d	2.32de	3.4b-d	3.18d-f
BA 1.0+ IBA 2.0	3.20ab	2.30ef	2.8d-f	3.08e-f
BA 2.0 + IBA 0.5	3.20a-c	3.14b	2.4e-h	4.08b
BA 2.0 + IBA 1.0	4.40a	2.54c	3.00c-d	4.40a
BA 2.0 + IBA 1.5	3.20a-c	3.12b	4.2a	4.10b
BA 2.0 + IBA 2.0	2.60b-e	3.66a	2.6e-g	3.74c
BA 3.0 + IBA 0.5	2.20d-g	2.16fg	2.4e-h	2.72c-f
BA 3.0 + IBA 1.0	2.20d-g	1.90gh	3.6a-c	2.50gh
BA 3.0 + IBA 1.5	2.40c-f	1.74hi	2.4e-h	2.29f-h
BA 3.0 + IBA 2.0	2.20e-g	1.56ij	2.2f-i	2.45hi
CV (%)	25.22	7.85	26.31	7.93
$LSD_{(0.05)}$	0.742	0.1855	0.7817	0.2534

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



(a) (b) (c)

(d) (e)

Fig. 1: *In vitro* regeneration of Strawberry: **(a)** Initiation of shoot **(b&c)** Shoot multiplication at 2.0 mg/l of BA and BA 2.0 mg/l + IBA 1.0 mg/l **(d&e)** Root formation in the treatment of 2.0 mg/l of IBA and BA 2.0 mg/l+ IBA 1.50 mg/l

(a) (b)

Fig. 2: Hardening of strawberry plantlet in **(a)** shade condition; and in **(b)** open condition

containing 1.0 mg/l BAP + 0.1 mg/l IBA, showed 90% shoot initiation and 5.5 ± 0.51 cm average length of shoot per explants in Strawberry. The results of our experiment were varied may be due to genotype and culture environment (Sen *et al.* 2002).

The treatment BA 2.0 mg/l + IBA 1.50 mg/l gave the highest number of root (4.20) at 28 DAI. The highest length of root (4.40 cm) at 28 DAI was found in BA 2.0 mg/l+ IBA 1.0 mg/l (Table 3). Mengxing *et al.* (2020) showed that better culture effect could be obtained by selecting the 5th generation of sub-cultured plantlets, adopting the inoculating density of 2 plants/bottle and intermittent immersion frequency of 10 min/L h and selecting the hormone combination of 3.0 mg/L BA + 0.01 mg / L NAA. Besides, shoot cultures were obtained from shoot tips on MS with 4% table sugar, 0.75% agar, 5 mg/ L BA and 0.01 mg/ L kinetin. These shoots were multiplied and maintained on MS medium with 1 mg/ L BA and 0.1 mg/ L kinetin (Dhukate *et al.* 2021).

Acclimatization and establishment of plantlets on soil

The regenerated plantlets of 6 to 8 weeks were transferred from cultural vial into small plastic pots prepared with a standard ratio of cowdung and soil in a shade condition. The water was sprayed onto the plantlets occasionally for maintaining humidity. The survival rate was 80% in shade condition and 75% in open air. So, the acclimatization potential of Strawberry was satisfactory. According to Dhukate *et al.* (2021) after four weeks, plantlets were transferred from controlled condition to the net-house onto plastic bags containing garden soil for acclimatization and hardening and then were transferred to fields.

CONCLUSION

The results of the present study indicated that BA 2.0 mg/l showed the good performance for shoot induction and IBA 1.5 mg/l and 2.0mg/l gave the best performance in case of root. In contrast MS medium supplemented with 2.0 mg/l BA+1.0 mg/l IBA showed the best response for shoot and root formation. The results showed that the combination of BA and IBA was better than BA alone in the basal MS medium *in vitro* regeneration. So, finally it can be concluded that, a convenient protocol of rapid regeneration of strawberry is established that will hold promise in developing disease resistant and climate adaptive cultivars, year round production, and ensuring genetic uniformity. These advancements can enhance commercial viability and meet the rising demand for high quality strawberries.

REFERENCES

Anuradha, S.K., Poonia, A.K., Kajla, S. and Bhat, S. 2016. Production of strawberry plants by *in vitro* propagation. *Res. Crops*, **17**(3): 545-549.

Anonymous. 2016. Commercial strawberry cultivation on the rise in Rajshahi. Retrieved from https://www.observerbd.com/2016/02/10/135849.php

Biswas, M.K., Dutt, M., Roy, U.K., Islam, R. and Hossain, M. 2009. Development and evaluation of *in vitro* somaclonal variation in strawberry for improved horticultural traits. *Sci. Hortic.*, **22**: 409-416.

Boxus, P. 1974. The production of strawberry plants by *in vitro* micropropagation. *J. Hortic. Sci.*, **49**: 209-210.

Capocasa, F., Balducci, F., Marcellini, M., Bernardini, D., Navacchi, O. and Mezzetti, B. 2019. Comparing nursery behavior, field plant yield and fruit quality of *in vitro* and *in vivo* propagated strawberry mother plants. *Plant Cell Tissue Organ Cult.*, **136**: 65-74.

da Fonseca, A.P., da Silva, E.C., Pereira, M.B., de Oliveira, R.P. and Dornelles, A.L.C. 2013. Phenotypic stability of strawberry genotypes subjected to variable number of *in vitro* subcultures. *Cienc. Rural*, **43**(8): 1345-1350.

Dhukate, M.R., Kher, M.M., Vadawale, A.V., and Giri, P. 2021. Protocol for micropropagation of strawberry (*Fragaria× ananassa*) cv. 'Sweet Charlie' and 'Winter Dawn'. *Environ. Exp. Biol.*, **19**: 1-6.

FAOSTAT. 2021. Retrieved from https://www.fao.org/faostat/en/#data/QCL/visualize

Giampieri, F., Alvarez-Suarez, J.M., Gasparrini, M., Forbes-Hernandez, T.Y., Afrin, S., Bompadre, S. *et al.* 2016. Strawberry consumption alleviates doxorubicin-induced toxicity by suppressing oxidative stress. *Food Chem. Toxicol.*, **94**: 128-137.

Jhajhra, S., Dashora, L.K., Singh, J., Bhatnagar, P., Kumar, A. and Arya, C.K. 2018. *In vitro* Propagation of Strawberry (*Fragaria × ananassa* Duch.). *Int. J. Curr. Microbiol. Appl. Sci.*, **7**(10): 3030-3035.

Kadhimi, A.A., Alhasnawi, A.N., Mohamad, A., Yusoff, W.M.W., Che, C.R.B. and Zain, M. 2014. Tissue culture and some of the factors affecting them and the micropropagation of strawberries. *Life Sci. J.*, **11**(8): 484-493.

Kaushal, K., Nath, A.K., Kaundal, P. and Sharma, D.R. 2004. Studies on somaclonal variation in strawberry (*Fragaria × ananassa* Duch.) cultivars. *Acta Hortic.*, **662**: 269-275.

Keiko, O., Shigeru, A. and Hiroshi, A. 2003. Effect of cytokinin on strawberry [Fragaria] plantlets micropropagated by axillary buds. *Bull. Nara Prefect. Agric. Exp. Stat.*, **34**: 15-24.

Labade, G.B., Dale, N.S., Umbarkar, R.B., Gadhe, S.K. and Rote, Y.N. 2016. In Vitro Regeneration of Strawberry (*Fragaria ananassa* D.). *Int. Inf. Res. Rev.*, **3**(11): 3043-3045.

Lawand, S., Patel, H., Rana, M. and Rajkumar, S. 2022. Effect of Explant Origin and Medium Plant Growth Regulators on *In Vitro* Shoot Proliferation and Rooting of *Salvia tomentosa*, a Native Sage of the Northeastern Mediterranean Basin. *Agronomy*, **12**(8): 1889.

Martin, R.R. and Tzanetakis, I.E. 2013. High risk strawberry viruses by region in the United States and Canada: implications for certification, nurseries, and fruit production. *Plant Dis.*, **97**: 1358-1362.

Mengxing, Lv, J., Zhang, X., Qiu, L., Ma, J., Ruan, Y. and Chen, Z. 2020. Optimization of the rapid propagation system for strawberry tissue culture seedlings through Temporary Immersion Bioreactors (Tibs). *Int. J. New Dev. Eng. Soc.*, **4**(1).

Naing, A.H., Kim, S.H., Chung, M.Y. *et al.* 2019. *In vitro* propagation method for production of morphologically and genetically stable plants of different strawberry cultivars. *Plant Methods*, **15**: 36.

Quiroz, K.A., Berríos, M., Carrasco, B., Retamales, J.B., Caligari, P.D.S. and García-Gonzáles, R. 2017. Meristem culture and subsequent micropropagation of Chilean strawberry (*Fragaria chiloensis* (L.) Duch.). *Biol. Res.*, **50**: 1-11.

Sen, J., Kalia, S. and Mukherjee, S.G. 2002. Level of endogenous free amino acid during various stages of culture of *Vigna mungo* (L) somatic embryogenesis, organogenesis and plant regeneration. *Curr. Sci.*, **82**(4): 429-433.

Singh, R. and Kumar, S. 2022. Rapid multiplication of strawberry (*Fragaria* × *ananassa* Dutch) by *in vitro* runner tip culture. *J. Emerg. Technol. Innov. Res.*, **9**(1).

Ulrich, D., Kecke, S. and Olbricht, K. 2018. What do we know about the chemistry of strawberry aroma. *J. Agric. Food Chem.*, **66**: 3291-3301.

Zahedi, S.M., Moharrami, F., Sarikhani, S. *et al.* 2020. Selenium and silica nanostructure-based recovery of strawberry plants subjected to drought stress. *Sci. Rep.*, **10**: 17672.