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In Vitro Propagation of Three Strawberry Varieties and Field Evaluation

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Abstract

A study was done to produce a rapid *in vitro* propagation of three strawberry genotypes and tested in the field under Bangladeshi circumstances. Festival, RABI-3, and Neho strawberry genotypes' runner tips were cultivated *in vitro* to induce root induction and multiple shoot proliferation. MS (Murashige and Skoog) media that were basally containing three different concentrations at 1.0, 1.5, and 2.0 of BA (6-benzyl adenine), KIN (6-furfuryl amino purine), or GA_3 (gibberellic acid) at 0.5 mg/L increasing tips of the runner was attained. The culture grew on the medium provided with 1.5 mg/L 6-benzyl adenine and 0.5 mg/L 6-furfuryl amino acid to increase shoot at the best level. Micro-cuttings were rooted on MS media at half strength combined with 0.5 mg/L - 1.5 mg/L IBA (indole butyric acid) or IAA (indole acetic acid). IBA attained 4 - 9 roots and 91% - 96% rooting at 1.0 mg/L. The resulting plantlets grew into hardy plants and took root in the earth. The genotype festival had the highest response rate, followed by RABI-3 and Neho.

Keywords

Strawberry, *In Vitro*, Propagation, Genotypes, Root Induction, Shoot Proliferation

1. Introduction

Strawberry is a little fruit plant that combines two highly diverse species named *Fragaria* × *ananassa* Duch., which are octoploids that have survived in a wide range of environmental circumstances [1]. The Rosaceae family includes this perennial stoloniferous plant. Strawberries are delicious, containing many nutrients, and millions of people appreciate strawberries worldwide in various climatic

conditions, including taiga, Mediterranean, subtropical, and temperate/modest areas [2]. Strawberry is the term for the edible, aggregate fruit, which is usually red when ripe.

The garden strawberry (*Fragaria* × *ananassa* and related cultivars) is the most popular worldwide strawberry. It is a cross between Chile's Fragaria chiloensis and eastern North American *Fragaria virginiana*, the main native-grown strawberry variety in the United States recognized for its large size and flavor, respectively. Strawberries can be cultivated on various soil types, from sandy to loamy, as long as they are well-drained and have a pH of 5 - 7. Because plants are frequently maintained for about 1 - 4 years and have shallow root systems, depth is not essential in most cultural systems. Strawberries contain many ellagic acids, which are phenolic flavon*o*ids and phytochemicals. Strawberry consumption has been shown in scientific research to offer possible health benefits against cancer, aging, inflammation, and neurological illnesses, increase HDL (good) cholesterol, lower blood pressure, and prevent congenital disabilities, including spina bifida. Strawberry plants are typically propagated through vegetative means that are rooted runners. However, this method has been proven unsuccessful due to several diseases and environmental dangers, which have resulted in a progressive fall in farmers' production. When compared to conventionally propagated runner plants, micro-propagated strawberry plants performed significantly better in terms of various characteristics (size of the crown, number of runners produced, and the amount of berries yield), according to Karhu and Hakala [3].

Traditionally, strawberry plants are multiplied by runners, which are labor and time-intensive and cause the spread of infectious diseases [4]. As a superior alternative to the conventional propagation approach, tissue culture has shown mass multiplication *in vitro* to produce disease-free plant components highly [5] [6]. Many have successfully tried standardizing the methodology and procedure of growing strawberries from micro-particles [7] [8] [9]. However, more research needs to be done on how well micro-propagated plants perform in the field, and a thorough field evaluation is required before tissue culture can be used commercially [10]. Additionally, the market's needs must be supplied by a variety of ways of production.

Introducing the micro-propagated strawberry plant aims to prevent most soil-transmitted illnesses [11]. The ideal photoperiod for better strawberry production is between 10 and 20 hours, daytime temperatures of 12˚C - 30˚C, and 12 - 24 short days per week [12]. Bangladesh belongs to a subtropical area, where wintertime temperatures range from 15˚C to 25˚C, the photoperiod is between 12 h - 16 h, and the day lasts between 30 and 50 days [11].

Strawberries can consequently be cultivated in the winter and are currently highly demanding because of the attractiveness of the fruits, aroma, and nutritional value. Strawberries have been produced in Bangladesh for a few years, but the country's extreme summer heat is their most significant obstacle to development. In the current study, we made an effort to create a workable method for strawberry plant reproduction *in vitro* for the mass amount of planting materials

production that can be effective for the production of strawberries commercially in Bangladesh.

2. Materials and Methods

Three varieties named Festival, RABI-3, and Neho strawberry (*Fragaria* × *ananassa* Dutch.) were selected for this research. The local variety explants named RABI-3 were obtained from the stocks grown in the Akafuzi Agrotechnologis field located at Rajshahi, Bangladesh. The strawberry germplasm stocks kept in the Plant Breeding and Gene Engineering Laboratory, Department of Botany, Rajshahi University, Bangladesh, were used to collect the Festival (American variety) and Neho varieties.

At the beginning of November 2019, Young tips from runners were gathered from mature strawberry plants that had been growing for two months. The three strawberry tips of the runner were repeatedly rinsed with distilled water after being cleaned for half an hour using running tap water and treated with 1% NaOCl. Additional sterilizing was carried out in an aseptic environment in a laminar airflow cabinet. The surface of explants was sanitized for 1 minute using a concentration of 50% (v/v) ethyl alcohol, then for an additional 4 minutes using a concentration of 0.1 percent (w/v) $HgCl_2$.

Then, the sterile deionized water was used five times for cleaning the explants, sized correctly at 1.5 cm. After that, explants were planted on a rudimentary medium of MS that is provided with particular concentrations of growth controllers, namely BA (6-benzyl adenine), KIN (6-furfuryl amino purine), GA_3 (gibberellic acid), and also 30 g/L market sugar mixing as well as 0.8% of agar. Before autoclaving, the medium's pH was maintained at 5.7 at 1.06 Kg/cm^3, providing heat of 121˚C for 20 minutes. A chamber for growth where the temperature was 25˚C ± 2˚C and a light period of 16 hours where the dark period was 8 hours for raising the prepared cultures.

Numerous proliferating shoots were divided into individual nodes. Then, the projections were placed in a medium of MS at half-strength with various amounts of IBA or IAA to initiate the root. The current experiment used reagent-grade chemicals from either MERCK, India, or BDH, England, including macro as well as micro-nutrients, organic and inorganic acids, agar, alkali like KOH, some salt like $HgCl_2$, sugar, ethanol, etc. British Drug House (BDH) in England produced a tiny amount of the vitamins, Glycin, growth regulators, and thiamine.

In contrast, Sigma Chemical Company and Phytotec in the United States had most of these substances. After initiating the culture for five weeks, shooting and rooting effectiveness data were collected. The trials were performed thrice, with 10 to 12 explants for each treatment. To regulate the moisture condition, rooted shoots that were three weeks old were transferred into containers made of plastic with compost that was mixed with garden soil at (1:3 v/v). After being withdrawn from the culture tubes, the shoots were thoroughly rinsed with water to remove the agar gel.

After a week, the plants were removed from the shed, and those that had survived were planted in the field. To assess the varietal outcomes of the seven strawberry genotypes, information on ten randomly chosen plants/varieties was collected, including information on the height of the plant, leaves number in each plant, stolons numbers in each plant, nodes number in each stolon, size of the canopy (cm^2), flowers number in each plant, fruits number in each plant, and fruits weight in each plant (g). After 80 days of plantation, the number of fruits/plants was recorded, and then, after 60 days, additional characters were registered.

3. Results

Different BA concentrations along the KIN or GA_3 effect for generating enormous shoots from tips of the runner of three strawberry genotypes are shown in Table 1.

Three different strawberry kinds' runner tips were inoculated on MS media that was provided with BA to varying concentrations at 1.0 mg/L, 1.5 mg/L, and 2.0 mg/L and KIN at various concentrations at 0.1 mg/L and 0.5 mg/L, or gibberellic acid at 0.1 mg/L and 0.5 mg/L. Directly from the explants, several shoots appeared within 8 to 14 days of culture. Explants grown in medium containing BA at 1.5 mg/L together with a concentration of GA_3 or KIN started beginning shoots 2 - 4 days earlier than those exposed to other treatments. The rate of shoot

Table 1. Produced effects of various BA concentrations along KIN or GA_3 for producing enormous shoots from runner tips of three strawberry genotypes.

Growth regulators conc. (mg/l)	Genotypes	% of explants showing shoot proliferation	Days to shoot formation	No. of shoots	Shoot length (cm)
1.0 mg/l BA + 0.5 mg/l KIN	Festival	77	10 - 11	3.00	3.3
	RABI-3	74	10 - 11	2.33	3.2
	Neho	72	10 - 11	2.00	3.0
1.5 mg/l BA + 0.5 mg/l KIN	Festival	98	8 - 10	5.67	3.2
	RABI-3	93	8 - 10	7.00	3.0
	Neho	90	8 - 10	2.67	3.1
2.0 mg/l BA + 0.5 mg/l KIN	Festival	87	10 - 11	3.00	3.2
	RABI-3	83	10 - 11	3.00	3.1
	Neho	78	10 - 11	2.00	3.2
1.0 mg/l BA + 0.5 mg/l GA_3	Festival	75	10 - 12	3.67	3.2
	RABI-3	72	10 - 12	5.00	3.2
	Neho	71	10 - 12	1.33	3.1
1.5 mg/l BA + 0.5 mg/l GA_3	Festival	95	8 - 10	3.00	3.1
	RABI-3	90	8 - 10	4.33	3.0
	Neho	85	8 - 10	2.67	2.9
2.0 mg/l BA + 0.5 mg/l GA_3	Festival	88	10 - 14	5.34	3.2
	RABI-3	81	10 - 12	3.33	3.1
	Neho	80	10 - 12	1.67	3.0

proliferation varied from 86% - to 98%, then the BA at 1.5 mg/L and KIN at 0.5 mg/L combination produced the highest rate of report for all genotypes (Table 1).

Shoot formation was the most frequent (98%) in the festival, followed by RABI-3 (93%) and Neho (90%). In BA at 1.5 mg/L and KIN at 0.5 mg/L, there were 6 - 7 shoots per explant, while in BA at 1.0 mg/L combined with GA_3 at 0.5 mg/L, there were 4 - 5 shoots per explant. Compared to other treatments, there were few roots per plant. The number of nodes/explants grew after the level of BA was increased from 1.0 mg/L - 1.5 mg/L, but as the BA concentration was raised further, the shot number declined. RABI-3 in 1.5 mg/L BA combined with 0.5 mg/L KIN produced the most shoots/explant, followed by Festival and Neho.

Table 2 effects of various IBA and IAA concentrations on the *in vitro* rooting of micro-shoots of three strawberry varieties. Data were recorded following a 5 weeks culture.

The incidence of adventitious root induction adjusted from 80% - 96%, and both IBA and IAA were found to be successful. When comparing IBA and IAA at two different concentrations, it was found that IBA performed better for inducing roots than IAA at 1.0 mg/L, where the shoots began to generate roots sooner. Festival developed the most roots in each shoot and also the most inductions of the root (96%) in media supplemented, including 1.0 mg/L IBA (Table 2).

During this experiment, there was no difference in the length of the roots. In Table 3 in the field, plantlets were transplanted 60 - 80 days later, and various agronomic characteristics of three strawberry genotypes after two months were observed. The findings demonstrated that plant development varied widely for all agronomic characteristics except plant height.

Plantlets that were rooted were removed from the tube of the culture, and to get rid of the material of the culture, they were rinsed thoroughly in tap water. In

Table 2. Different IBA and IAA concentrations affect on rooting by *in vitro* technique of micro-shoots of three strawberry types.

Growth regulators conc. (mg/l)	Genotypes	% of micro-cuttings rooted	Days to root formation	No. of roots/ Micro-cutting	Root length (cm)
MS + 0.5 mg/l IBA	Festival	85	12 - 14	7.33	2.4
	RABI-3	83	10 - 12	6.47	2.3
	Neho	80	10 - 12	3.33	2.0
MS + 1.0 mg/l IBA	Festival	96	12 - 14	8.67	2.6
	RABI-3	92	10 - 12	4.33	2.5
	Neho	91	10 - 12	3.67	2.2
MS + 0.5 mg/l IAA	Festival	83	8 - 10	4.56	2.3
	RABI-3	82	8 - 11	3.33	2.2
	Neho	80	8 - 10	2.67	2.0
MS + 1.0 mg/l IAA	Festival	90	10 - 12	2.63	2.2
	RABI-3	88	10 - 12	3.55	2.1
	Neho	87	10 - 12	2.87	2.0

Table 3. Three strawberry genotypes' micro-propagated plantlets responded in the field.

Genotypes	Plant height (Mean ± SE)	No. of leaves/plant (Mean ± SE)	No. of stolons/plant (Mean ± SE)	Stolon length (cm) (Mean ± SE)	Canopy size (cm^2) (Mean ± SE)	No. of flower/plant (Mean ± SE)	No. of fruits/plant (Mean ± SE)	Fruit wt./plant (Mean ± SE)
Festival	16.13 ± 0.02	15.2 ± 0.58	4.4 ± 0.51	100.118 ± 0.07	385.33 ± 0.02	12.60 ± 0.51	6.20 ± 0.37	153.55 ± 0.26
RABI-3	15.39 ± 0.02	12.8 ± 0.37	4.6 ± 0.51	107.524 ± 0.29	344.99 ± 0.02	14.00 ± 0.44	6.00 ± 0.71	142.41 ± 0.22
Neho	13.12 ± 0.04	13.2 ± 0.58	3.8 ± 0.37	117.772 ± 0.07	417.63 ± 0.11	13.60 ± 0.51	4.20 ± 0.37	133.79 ± 0.17

a thump pot, cleaned plantlets were planted after being sprayed with fungicide and placed in regular, sterile soil. The hardened plantlets were buried in the ground after seven days. Plant height (16.13 ± 0.02), leaf count (15.2 ± 0.58), fruit count (6.20 ± 0.37), and fruit weight (153.55 ± 0.26) were the festival's best performers. The number of stolons per plant (4.6 ± 0.51) and the number of flowers per plant (14.00 ± 0.44) were where RABI-3 excelled. Stolon length (117.772 ± 0.07) and canopy size (417.63 ± 0.11) are where Neho performed best.

Neho had the lowest plant height, stolons per plant, fruit production, and fruit weight per plant (13.12 ± 0.04, 3.8 ± 0.37, 4.20 ± 0.37, and 133.79 ± 0.17), respectively. RABI-3 had the lowest number of leaves per plant (12.8 ± 0.37) and the largest canopy size (344.99 ± 0.02). The Festival region has the fewest flowering plants (12.6 ± 0.51) and the lowest stolon length (100.118 ± 0.07). Festival in this investigation was found to be more sensitive than the other two types, both *in vitro* and *ex vivo*.

4. Discussion

Analyzing Table 1, explants grown in the prepared media containing BA at 1.5 mg/L together with KIN or GA_3 began shoots 2 - 4 days earlier than those treated with other therapies. The combination of BA at 1.5 mg/L and KIN at 0.5 mg/L provided the best result report for all genotypes, with a range of shoot proliferation rates between 86% and 98% (Table 1). Formation of shoot found at maximum speed at the festival (98%), followed by RABI-3 (93%) and Neho (90%).

There were 6 - 7 shoots per explant in BA at 1.5 mg/L combined with KIN at 0.5 mg/L, compared to 4 - 5 nodes per explant in BA at 1.0 mg/L combined with GA_3 at 0.5 mg/L GA_3. There were fewer roots per plant compared to other treatments. It was found that when the BA concentration increased from 1.0 mg/L - 1.5 mg/L, the shoot number increased, but as the BA concentration increased further, the shoot number decreased. RABI-3 in BA at 1.5 mg/L and KIN at 0.5 mg/L produced the most shoots/explant, followed by Festival and Neho.

According to Hu and Wang [13], there were fewer micro-propagated shoots when cytokinin concentrations were high. There have already been reports of comparable outcomes in *Fragaria indica* Andr [14]. Several researchers reported strawberry shoot regeneration utilizing MS medium involving BA and KIN [15]

[16] [17] [18] [19]. Our findings showed that modest BA concentrations alone or combined with KIN were compatible with the start of new shoots and subsequent multiplication.

This variance could be caused by the explants' genotype and physiological condition. Daughter shoots (diameter 3 - 4 cm) were removed and placed in root initiation media. Indole butyric acid and IAA were found to be effective, and the prevalence of adventitious root induction increased from 80% to 96%. The results showed that IBA worked better for inducing roots than IAA at 1.0 mg/L, where the shoots started to create roots more quickly. In media supplemented with 1.0 mg/L IBA, festival produced the most roots per shoot and the most root inductions (96%) (**Table 2**).

There was no difference in the roots' length throughout this experiment. IBA also has a comparable impact on *Prunus* sp. [20], *Capsicum annum* [21], and *Calotropis gigentea* [22]. Festival in this study was discovered to be both more sensitive *in vitro* and *ex vivo* (**Table 3**) than the other two types. The method described here is repeatable and has the potential to allow Bangladesh to multiply this significant and novel fruit plant on a vast scale.

5. Conclusion

Proliferation of runner tips was achieved on Murashige and Skoog (MS) media that were basally containing three different doses at 1.0 mg/L, 1.5 mg/L, 2.0 mg/L of BA combined with KIN at 0.5 mg/L or GA_3 at 0.5 mg/L. Medium where cultures were grown provided with BA at 1.5 mg/L and 0.5 mg/L KIN demonstrated the highest shoot proliferation. MS media at half-strength with 0.5 mg/L - 1.5 mg/L of IBA or the same concentrated IAA. Micro-cuttings were planted. IBA attained 4 - 9 roots and 91% - 96% rooting at 1.0 mg/L. The resulting plantlets grew into hardy plants and took root in the earth. The genotype festival had the highest response rate, followed by RABI-3 and Neho.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Rieger, M. (2005) Strawberry. In: Rieger, M., Ed., *Introduction to Fruit Crops*, Haworth Food & Agricultural Products Press, New York, 383-392.

[2] Hancock, J.F., Mass, J.L., Shanks, C.H., Breen, P.J. and Luby, J.J. (1991) Strawberry (*Fragaria*). *Acta Horticulturae*, **290**, 491-548. https://doi.org/10.17660/ActaHortic.1991.290.11

[3] Karhu, S. and Hakala, K. (2002) Micropropagated Strawberries on the Field. *Acta Horticulturae*, **567**, 321-324. https://doi.org/10.17660/ActaHortic.2002.567.68

[4] Gautam, H., Kaur, R., Sharma, D.R. and Thakur, N. (2001) A Comparative Study on *in Vitro* and *ex Vitro* Rooting of Micropropagated Shoots of Strawberry (*Fragaria* × *ananassa*). *Plant Cell Biotechnology and Molecular Biology*, **2**, 149-152.

[5] Mahajan, R., Kaur, R., Sharma, A. and Sharma, D.R. (2001) Micropropagation of Strawberry Cultivar Chandler and Fern. *Crop Improvement*, **28**, 19-25.

[6] Mohan, R., Chui, E.A., Biasi, L.A. and Soccol, C.R. (2005) Alternative *in Vitro* Propagation: Use of Sugarcane Bagasse as a Low Cost Support Material during Rooting Stage of Strawberry cv. Dover. *Brazilian Archives of Biology and Technology*, **48**, 37-42. https://doi.org/10.1590/S1516-89132005000400005

[7] Kaur, R., Goutam, H. and Sharma, D.R. (2005) A Low Cost Strategy for Micropropagation of Strawberry (*Fragaria* × *ananassa* Duch.) cv. Chandler. *Acta Horticulturae*, **696**, 129-133. https://doi.org/10.17660/ActaHortic.2005.696.22

[8] Sakila, S., Ahmed, M.B., Roy, U.K., Biswas, M.K., Karim, R., Razvy, M.A., Hossain, M., Islam, R. and Hoque, A. (2007) Micropropagation of Strawberry (*Fragaria x ananassa* Duch.) a Newly Introduced Crop in Bangladesh. *American-Eurasian Journal of Scientific Research*, **2**, 151-154.

[9] Gantait, S., Mandal, N., Bhattacharyya, S. and Das, P.K. (2010) Field Performance and Molecular Evaluation of Micropropagated Strawberry. *Recent Research in Science and Technology*, **2**, 12-16.

[10] Smith, M.K. and Hamill, S.D. (1996) Filed Evaluation of Micropropagated and Conventionally Propagated Ginger in Subtropical Queensland. *Australian Journal of Experimental Agriculture*, **36**, 347-354. https://doi.org/10.1071/EA9960347

[11] Biswas, M.K., Islam, R. and Hossain, M. (2008) Micropropagation and Field Evaluation of Strawberry in Bangladesh. *International Journal of Agricultural Technology*, **4**, 167-182.

[12] Verheul, M.J., Sønsteby, A. and Grimstad, S.O. (2006) Interactions of Photoperiod, Temperature, Duration of Short-Day Treatment and Plant Age on Flowering of *Fragaria* × *ananassa* Duch. cv. Korona. *Scientia Horticulturae*, **107**, 164-170. https://doi.org/10.1016/j.scienta.2005.07.004

[13] Hu, C.Y. and Wang, P.J. (1983) Meristem Shoot Tip and Bud Culture. In: Evans, *et al.*, Eds., *Hand Book of Plant Tissue Culture*, Macmillan, New York, 177-227.

[14] Bhatt, I.D. and Dhar, U. (2000) Micropropagation of Indian Wild Strawberry. *Plant Cell, Tissue and Organ Culture*, **60**, 83-88. https://doi.org/10.1023/A:1006471815566

[15] Lee, E.C.M. and Fossard, R.A. (1977) Some Factors Affecting Multiple Bud Formation of Strawberry (*Fragaria* × *ananassa* Duch.) *in Vitro*. *Acta Horticulturae*, **78**, 187-196. https://doi.org/10.17660/ActaHortic.1977.78.24

[16] Sobczykiewicz, D. (1980) Heat Treatment and Meristem Culture for the Production of Virus-Free Strawberry Plants. *Acta Horticulturae*, **95**, 79-82. https://doi.org/10.17660/ActaHortic.1980.95.10

[17] Lis, E.K. (1990) *In Vitro* Clonal Propagation of Strawberry from Immature Achenes. *Acta Horticulturae*, **280**, 147-150. https://doi.org/10.17660/ActaHortic.1990.280.24

[18] Neeru, S., Ranjan, S., Singh, O.S. and Gosal, S.S. (2000) Enhancing Micropropagation Efficiency of Strawberry Using Bandage in Liquide Media. *Journal of Applied Horticulture*, **2**, 92-93. https://doi.org/10.37855/jah.2000.v02i02.27

[19] Mereti, M., Grigoriadou, K., Levantakis, N. and Nanos, G.D. (2003) *In Vitro* Rooting of Strawberry Tree (*Arbutus unedo* I.) in Medium Solidified by Peat-Perlite Mixure in Combination with Agar. *Acta Horticulturae*, **616**, 207-210. https://doi.org/10.17660/ActaHortic.2003.616.25

[20] Mante, S., Scorza, R. and Cordts, J.M. (1989) Plant Regeneration from Cotyledons of *Prunus persieg*, *P. domestica* and *P. cerasus*. *Plant Cell, Tissue and Organ Culture*,

19, 1-11. https://doi.org/10.1007/BF00037771

[21] Agarwal, S., Chandra, N. and Kothari, S.L. (1989) Plant Regeneration and Tissue Culture of Pipper (*Capsicum annum* L. cv. Mathania). *Plant Cell*, *Tissue and Organ Culture*, **16**, 47-55. https://doi.org/10.1007/BF00044071

[22] Roy, A.T. and De, D.N. (1986) *In Vitro* Plantlets Regeneration of the Petrocrop *Calotropis gigantia*. Bioenergy Society of India, New Delhi, 123-128.