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In Vitro Propagation of Three Strawberry Cultivars through Runner Tips Culture

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Abstract

At the Genetics and Plant Breeding Laboratory of the Department of Agronomy and Agricultural Extension, University of Rajshahi, Bangladesh, strawberry *in vitro* propagation was done. Five Benzylaminopurine (BAP) concentrations were utilized for shoot induction—0.0 mg/L (Control), 0.5 mg/L, 1.0 mg/L, 1.5 mg/L, and 2.0 mg/L and five Indole Buteric Acid (IBA) concentrations—0.0 mg/L(Control), 0.5 mg/L, 1.0 mg/L, 1.5 mg/L, and 2.0 mg/l—were used for the induction of the root. The highest mean amount of shoots (eight) and length of the shoot (3.40 cm) were observed at a concentration of BAP of 0.5 mg/L. Festival also had the highest mean amount of leaves (6) when we used the identical concentration, while RABI-3 and Camarosa did the least well. The IBA of 0.5 mg/L concentration of rooting performed the best across all metrics tested among the five concentrations. The longest (3.3 cm) roots and most roots (7) were likewise obtained from this concentration in Festival. However, RABI-3 and Camarosa performed worse than Festival in the same concentration. Half-strength MS media without IBA concentration showed no response regarding root induction for each of the three cultivars.

Keywords

Strawberry, Proliferation, Propagation, Shoot Induction, Root Induction

1. Introduction

Fragaria and *Ananassa* Duch., two incredibly diverse species that have thrived in various environmental conditions, were crossed to create the little fruit plant known as the strawberry [1]. This perennial plant that is stoloniferous belongs to the *Rosaceae* family. Millions of people worldwide appreciate strawberries in various climatic conditions, including the taiga, Mediterranean, sub-tropical, and tem-

perate zones, because they are sweet and healthful [2].

Ellagic acid, which is a phenolic flavonoid phytochemical, is abundant in strawberries. According to scientific studies, eating strawberries may help protect against diseases including cancer, aging, inflammation, and neurological disorders, as well as raise HDL (high-density lipoprotein), which is good cholesterol levels and blood pressure and prevents congenital disabilities like spina bifida. Although propagating cells produced from runners are estimated to make up 90% of the Dutch strawberry supply, plant propagation via runners, which is used in several species of the genus, only produces a modest amount of propagating cells.

It has been demonstrated that strawberry micropropagation accelerates the rate at which virus- and disease-free plant material may be made. Furthermore, plants grown from tissue culture produce more runners than plants grown through conventional propagation techniques [3]. In the culture medium, previous studies have shown that auxin concentrations, as well as the number of subcultures, are crucial factors in determining whether to include somaclonal variation in an *in vitro* system [4]. In commercial strawberry micropropagation, neither somatic embryogenesis nor shoot organogenesis is often used since they may result in somaclonal diversity in adventitiously regenerated plants.

Plant tissue culture techniques and somaclonal variety can generate whole new plants from various explants. Genetic diversity and callus culture regeneration have long been linked [5]. Conventional propagation techniques may not be advised for efficient and profitable multiplication because they are laborious, expensive, and slow [6]. Fast multiplication rates are one benefit of *in vitro* propagation [7]. Micropropagation has many benefits because it allows for the rapid and efficient production of numerous plants from a single individual. The current research was done with the facts above in mind [8].

However, more research needs to be done on how well micropropagated plants perform in the field, and a thorough field evaluation is required before tissue culture can be used commercially [9]. Additionally, more than the standard production method is required to satisfy the market requirement. 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 work, a substantial technique for strawberry *in vitro* plant regeneration was developed to produce large quantities of planting materials for the production, which is commercial in Bangladesh.

2. Materials and Method

The experiment was conducted from March to September 2019 at the Genetics and Plant Breeding Laboratory of the Department of Agronomy and Agricultural Extension, University of Rajshahi, Bangladesh. Five BAP concentrations—0.0 mg/L (Control), 0.5 mg/L, 1.0 mg/L, 1.5 mg/L, and 2.0 mg/L were applied aim to start induction of the shoot (**Figures 1-3**).

Five IBA concentrations—0.0 mg/L (Control), 0.5 mg/L, 1.0 mg/L, 1.5 mg/L, and 2.0 mg/L were used for the induction of root. In both the shoot and root initiation experiments, numerous shoots and roots, the mean number was calculated for each treatment, composed of one test tube and reproduced five times.

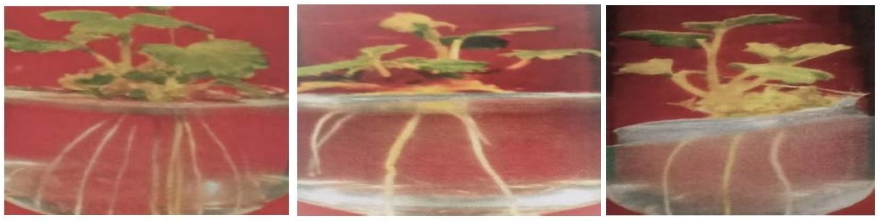
Typically, salts, both organic and inorganic, are a source of carbon, a few vitamins, and iron, as well as regulators for growth, make up a nutritional medium for plant regeneration. In this work, the base medium for plant regeneration was Murashige and Skoog (MS), 1962 medium. Festival, RABI-3, Camarosa runner segments and runner tips were employed as explants. In Rajshahi, Bangladesh, explants were collected from field-grown stocks owned by Akafuzi Agrotechnology. Only the delicate and continuously developing segments of the runner and tips of the runner that were prepared to be used as explants had a length of 1.00 - 1.25 cm.

(a) (b) (c)

Figure 1. Shoot proliferation of three cultivars at 0.5 mg/l BAP concentration: (a) Festival, (b) RABl-3, (c) Camarosa.

(a) (b) (c)

Figure 2. Multiple shoot of three cultivars was observed at 0.5 mg/l BAP concentration: (a) Festival, (b) RABl-3, (c) Camarosa.



(a) (b) (c)

Figure 3. Root induction of three cultivars was observed at 0.5 mg/l lBA: (a) Festival, (b) RABl-3, (c) Camarosa.

The explants were sterilized with Trix and $HgCl_2$ and washed with running tap water. Every step of the construction process included safety measures to maintain aseptic conditions. A laminar airflow cabinet was used for all injections and aseptic procedures. Throughout work, 70% ethyl alcohol was often applied to the hands and cabinet base to keep them clean. Explant preparation was done carefully to ensure a clean bench and possibly contamination-free conditions were reached. Inside the laminar airflow cabinet, the shoot tips were prepared with a scalpel and a fine pair of sterile forceps. The excised shoot tips were then inserted into the test tubes in medium containers containing various concentrations of BAP to facilitate the *in vitro* regeneration of the multiple shoots.

The physical conditions required for the culture's growth and development were maintained at 25˚C ± 1 ˚C and 2000 - 3000 lux, as provided by fluorescent tubes. The light period was maintained at light of 16 hours and darkness of 8 hours (16 L/8D), while relative humidity was between 60% and 70%. Once tiny, newly formed green leaves appeared, the shoots had been successfully created. It represents the start of regeneration. Once fully developed, these little leaves were transplanted into new media with the same hormone content to promote further growth and development of shoots. After the start of the shoot, the first and second subcultures were conducted at three and five weeks, respectively.

After being carefully extracted from the test tubes, the numerous branches that had been revitalized were laid out on sterile, stiff paper. The basal end of each shoot was clipped off and then moved to fresh media with BAP concentrations for additional induction of the multiple nodes. A half-strength amplified MS medium containing different IBA doses was used to induce root growth. The explant's number cultured, the average shoot number in each culture, the average shoot length in each culture, the number of moderately sized shoots in each culture, the number of days until root induction, the average roots number in each culture, and the average root length were all recorded. Mean data were derived to display the statistical information.

3. Results

3.1. Shoot Induction

The growth of runner segments and tips was demonstrated in Table 1 using MS media supplemented with different 6-Benzylamino purine concentrations: 0.0 mg/L (Control), 0.5 mg/L, 1.0 mg/L, 1.5 mg/L, and 2 mg/L. After three weeks, explants were subcultivated.

At a BAP concentration of 0.5 mg/L in Festival, the highest average shoots number (8), shoot length (3.40 cm), and leaves number (6) were observed. No shoots were produced at the highest BAP concentration (2.0 mg/l). The highest average shoot number, leaves, and length of the shoot were seen in RABI-3 and Camarosa at the BAP concentration of 0.5 mg/L (Table 1). Still, for all three varieties, no node was generated at the maximum BAP concentration of 2.0 mg/L. The remaining treatments yielded numerous weak shoots. In that order, the

Table 1. Shows the effects of varying BAP concentrations added to MS medium on the proliferation of explant shoots from three distinct strawberry cultivars.

Cultivars	BAP (mg/l)	No. of explants/ culture	Average no. of shoots/culture	Average length of shoots/ culture (cm)	Average no. of leaves/culture
Festival	0.0	10	2	0.64	3
	0.5	10	8	3.40	6
	1.0	10	4	2.5	3
	1.5	10	3	2.1	2
	2.0	10	0	0.0	0.0
RABI-3	0.0	10	2	0.57	2
	0.5	10	6	2.77	5
	1.0	10	3	2.2	2
	1.5	10	2	1.8	1
	2.0	10	0	0.0	0.0
Camarosa	0.0	10	2	0.55	2
	0.5	10	6	2.89	5
	1.0	10	2	2.00	2
	1.5	10	2	1.5	1
	2.0	10	0	0.0	0.0

mean shoot lengths in Festival, RABI-3, and Camarosa were 0.64, 0.57, and 0.55 cm, but in BAP-free media, the average number of leaves was 3, 2 and 2, respectively.

3.2. Root Induction

After five weeks, Table 2 shows that the expanded shoots were placed in the growing media. IBA (indole 3-butyric acid) performed best among the doses tested at 0.5 mg/l in all evaluated parameters.

IBA at concentrations of 0.5 mg/L and 1.0 mg/L took the least time (8 - 10 days) to induce root growth. At the same attention, Festival also had the most significant cultures number (7) and the most excellent length of roots (3.3 cm). Moreover, the most significant culture number (6) and the most excellent length of sources (3.0 cm) were obtained from the same concentration in RABI-3. In addition, the most significant number of roots/culture (5) and the longest seeds (3.0 cm) were obtained from the same concentration in Camarosa. On the other hand, the above three cultivars' roots produced by other treatments were narrow. In the control treatment, no sources were generated.

4. Discussion

Explants taken from the mother plant's active development are typically the easiest way to introduce cultures. In temperate locations, spring to early summer is often

Table 2. Three strawberry cultivars' capacity to stimulate root growth in response to varying IBA concentrations in half-strength MS medium cultivars.

Cultivars	Treatment IBA (mg/l)	Days of root initiation	Average number of roots/culture	Average length of roots/culture (cm)
Festival	0.0	-	-	-
	0.5	8 - 10	7	3.3
	1.0	8 - 10	5	2.6
	1.5	10 - 12	2	1.9
	2.0	10 - 12	2	1.1
RABI-3	0.0	-	-	-
	0.5	8 - 10	6	3.0
	1.0	8 - 10	4	2.3
	1.5	10 - 12	2	1.6
	2.0	10 - 12	1	1.0
Camarosa	0.0	-	-	-
	0.5	8 - 10	5	3.0
	1.0	8 - 10	4	2.0
	1.5	10 - 12	2	1.33
	2.0	10 - 12	1	1.0

the ideal period to harvest explants; on the other hand, there have been numerous documented exceptions to this rule.

There have been several studies about strawberry tissue culture published. According to Mahmood *et al.* [10], the medium enriched with BAP of 0.5 mg/L concentration showed an excellent shooting response in line with the current findings.

Nevertheless, there were eight leaves, which is inconsistent with the available data, which only indicates five leaves. In their study, El-Sayed *et al.* [11] examined the effects of BAP on strawberry multiplication *in vitro*. They discovered that shoot proliferation was better with a lower concentration of BAP (0.5 mg/L) than with BAP at doses ranging from 1.0 mg/L - 3.0 mg/L. The current investigation shows the same outcomes at greater BAP concentrations (2.0 mg/L). At various BAP levels, variations in plantlet growth stages were also seen. Three to four weeks after plantlets were injected with growing culture by Maliaricikova and Mokra, the formation of roots was detected [12].

Furthermore, Asahira and Kano achieved the same results in 1977 [13]. Root development was noticed two weeks after plantlet inoculation, which meant that these results did not have any similarities with the current findings. Boxus [14] observed that strawberry shoot tips cultivated on a mixture containing BA of 0.5 $mg{\cdot}dm^{-3}$, GA of 0.1 $mg{\cdot}dm^{-3}$, and 6.4 $g{\cdot}dm^{-3}$ agar multiplied the number of shoots. Still, Lal *et al.* [15] also reported on the significance of BA in strawberry shoot

production and found that BAP at 4.0 mg/L enriched MS medium of growth produced the most shoots in each explant.

The optimal root response was observed in IBA at 0.5 mg/L enriched media, whereas there was no rooting in a medium free from auxin. As a consequence of this effort, a strawberry micropropagation protocol was developed. However, for better advancement, this discovery could be applied to studies of plant genetic modification. Based on the effects of IBA on root response, the treatments with the lowest root reaction were IBA together with 0.5 mg/L, 1.0 mg/L, and 1.5 mg/L concentrations. These results resemble those that Emara [16] previously reported in specific ways. A WHO study on the effect of the strength of MS on root length showed that full MS was associated with the highest adequate record, while the other powers had less influence. According to Kaushal *et al.* [17], strawberries were rooted at half the strength of MS using a concentration of IBA at 1.0 mg/L.

5. Conclusions

Plantlet generation during propagation is significantly impacted by the number of shoots generated during the setting-up stage of culture, which also influences the number of nodes produced during the shoot multiplication stage. The current study uses runner-tip explants grown on various shoot-induction media to examine strawberry (*Fragaria* × *ananassa* Duch.) shoot production at the location of culture establishment. The culture media known as "shoot induction medium" contains plant growth regulators of the cytokinin type in larger ratios than those of the auxin type.

The research's main goal was to find the best medium for producing shoots from runner-tip culture at the early stages of culture establishment. Five BAP concentrations were utilized for the successful induction of shoots: 0.0 mg/L (Control), 0.5 mg/L, 1.0 mg/L, 1.5 mg/L, and 2.0 mg/L; 5 IBA concentrations aim to start the commencement of roots: 0.0 mg/L (Control), 0.5 mg/L, 1.0 mg/L, 1.5 mg/L, and 2.0 mg/L were applied. At a BAP concentration of 0.5 mg/L, the highest average number of the shoot (8) and length of the node (3.40 cm) were measured.

Festival also had the highest average number of leaves (6) at the same concentration, whereas RABI-3 and Camarosa had the worst performance. IBA at the rate of 0.5 mg/L outperformed the other four rooting concentrations in every metric tested. Festival produced the longest (3.3 cm) and most (7) roots from this concentration, although RABI-3 and Camarosa fared worse than Festival in the same attention. Half-strength MS media without the awareness of IBA had no effect on root induction for any of the three cultivars.

Conflicts of Interest

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

References

[1] Rieger, M. (2006) Introduction to Fruit Crops. CRC Press, Boca Raton. https://doi.org/10.1201/9781482298055

[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] Swartz, H.J., Galletta, G.J. and Zimmerman, R.H. (1981) Field Performance and Phenotypic Stability of Tissue Culture-Propagated Strawberries. *Journal of the American Society for Horticultural Science*, **106**, 667-673. https://doi.org/10.21273/JASHS.106.5.667

[4] Gaafar, R.M. and Saker, M.M. (2006) Monitoring of Cultivars Identity and Genetic Stability in Strawberry Varieties Grown in Egypt. *World Journal of Agricultural Sciences*, **2**, 29-36.

[5] Popescu, A.N., Isac, V.S., Coman, M.S. and Radulescu, M.S. (1997) Somaclonal Variation in Plants Regenerated by Organogenesis from Callus Culture of Strawberry (*Fragaria* × *ananassa*). *Acta Horticulturae*, **439**, 89-96. https://doi.org/10.17660/ActaHortic.1997.439.8

[6] Dhar, M. (1998) Techniques of Vegetative and *in Vitro* Propagation of Jackffilit. Ph.D. Thesis, Banggabandhu Shaikh Mujibar Rahman Agricultural University, Salna, 120.

[7] Conger, B.V. (1981) Principles and Practices of Cloning Agricultural Plants via *in Vitro* Techniques. CRC Press, Boca Raton, FL.

[8] Maharjan, S., Pradhan, S., Thapa, B.B. and Pant, B. (2019) *In Vitro* Propagation of Endangered Orchid, *Vanda pumila* Hook.f. through Protocorms Culture. *American Journal of Plant Sciences*, **10**, 1220-1232. https://doi.org/10.4236/ajps.2019.107087

[9] 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

[10] Mahmood, S., Rashid, H., Quraishi, A., Iqbal, N., Arjumand, S.S. and Malik, M.N. (1994) Clonal Propagation of Strawberry through Tissue Culture. *Pakistan Journal of Agricultural Research*, **15**, 54-59.

[11] El-Sayed, S.F., El-Sawy, A.M., Taha, S.S. and Gomah, M.Sh. (2017) Effect of Benzylaminopurine Concentration and Number of Subcultures on Behavior of Some Strawberry Cultivars *in Vitro*. *Egyptian Journal of Plant Breeding*, **21**, 1-12. https://doi.org/10.12816/0046534

[12] Maliaricikova, V. and Mokra, A. (1986) Clonal Propagation of Strawberry *in Vitro*. *Vedecke Prace Vyskumneho Ustavu Ovocnych a Okrasnych Drevin v Bojniciach*, **6**, 117-123.

[13] Asahira, T. and Kano, Y. (1977) Shoot Formation from Cultured Tissue of Strawberry Fruits. *Journal of the Japanese Society for Horticultural Science*, **46**, 317-324. https://doi.org/10.2503/jjshs.46.317

[14] Boxus, P. (1999) Micropropagation of Strawberry via Axillary Shoot Proliferation. In: Hall, R.D., Ed., *Plant Cell Culture Protocols*, Vol. 111, Humana Press Inc, Totowa, NJ, 103-114. https://doi.org/10.1385/1-59259-583-9:103

[15] Lal, M., Sharma, S. and Hegde, M.V. (2003) Micropropgation of Strawberry (*Fragaria* × *ananassa* Duch.). *Indian Journal of Horticultural Research*, **37**, 231-234.

[16] Emarah, H. (2008) Factors Affecting Propagation of Strawberry *(Fragaria* spp.) through Tissue Culture Techniques. *Journal of Producttivity and Development*, **13**, 191-212. https://doi.org/10.21608/jpd.2008.44837

[17] Kaushal, K., Nath, A.K. and Sharma, D.R. (2006) Establishment of Callus Cultures and Plant Regeneration in Strawberry (*Fragaria* × *ananassa* Duch.) Cv. Chandler. *Indian Journal of Plant Physiology*, **11**, 136-144.