Journal of Scientific and Innovative Research 2024; 13(3): 43-48
Available online at: www.jsirjournal.com

Research Article

ISSN 2320-4818
JSIR 2024; 13(3): 43-48

Received: 01-07-2024
Accepted: 29-09-2024
DOI: 10.31254/jsir.2024.13302

Kay Thi Oo
Department of Biotechnology Research, Tasoe Road, Kyaukse City, Mandalay Region, Myanmar

Kyaw Swar Oo
Department of Biotechnology Research, Tasoe Road, Kyaukse City, Mandalay Region, Myanmar

Mya Yadanar Htwe
Department of Biotechnology Research, Tasoe Road, Kyaukse City, Mandalay Region, Myanmar

Win Thein Htet
Department of Biotechnology Research, Tasoe Road, Kyaukse City, Mandalay Region, Myanmar

Lone Kyi
Department of Biotechnology Research, Tasoe Road, Kyaukse City, Mandalay Region, Myanmara

Wah Wah Win
Department of Biotechnology Research, Tasoe Road, Kyaukse City, Mandalay Region, Myanmar

Wah Wah San
Department of Biotechnology Research, Tasoe Road, Kyaukse City, Mandalay Region, Myanmar

Correspondence:
Dr. Kay Thi Oo
Director, Plant Tissue Culture Laboratory, Department of Biotechnology Research, Kyaukse Township, Mandalay Region, Myanmar
Email: kaythi123@gmail.com

An Efficient Protocol for *In vitro* Propagation of Strawberry (*Fragaria x ananassa*) through Nodal Segment Culture with the Use of Plant Growth Regulator (BAP)

Kay Thi Oo, Kyaw Swar Oo, Mya Yadanar Htwe, Win Thein Htet, Lone Kyi, Wah Wah Win, Wah Wah San

Abstract

Strawberry (*Fragaria x ananassa*) is grown for its superior culinary and nutritional worth in many regions of the world. The objective of this research was to develop an affordable, dependable, and effective procedure for strawberry plant *in vitro* multiplication. Typically, strawberries are vegetatively grown by runners, which makes it impossible to meet the current need for planting material. A successful micropropagation strategy for the large-scale generation of strawberry plantlets of a local cultivar was attempted to be developed in the current study. Evaluations have been conducted on the effects of varying doses of BAP (6-Benzyl amino purine) alone on shoot strawberry proliferation. It has been demonstrated that the highest number of shoots / explants have been produced in MS media at a concentration of 3 mg/L BAP (13.2). However, in contrast to all previous BAP treatments, the greater shoot length rose to 0.5 cm when 1.5 mg/L of BAP was included in the nutritional medium. The MS medium supplemented with 0.5 mg/L of BAP had the greatest number of leaves per explant (24.4). The average number of shoots per explant and root production were found to be satisfactorily affected by the alone concentration of BAP. The rooted plantlets that had survived were carefully moved into a plant growth room with regulated environmental conditions for hardening after being gradually acclimated.

Keywords: Nodal segment, *Fragaria x ananassa*, strawberry, BAP, *in vitro*.

INTRODUCTION

The strawberry (*Fragaria x ananassa*), a popular fruit and member of the Rosaceae family, is a dicotyledonous, perennial, stoloniferous plant that grows in most agricultural parts of the world [1]. One of the first fruits to be widely grown commercially in numerous countries worldwide was the strawberry. Strawbery (*Fragaria x ananassa*) cultivars are a valuable source of nutrients because they are good food supplies, tasty potassium, ascorbic acid, fiber, and simple sugar sources that may be converted into energy and other secondary metabolites [2]. Because of its flavor, taste, freshness, freezing, and processing industry, strawberries have long been considered a popular and tasty fruit [3]. They are also rich in protein, calcium, potassium, iron, copper, and B vitamins, B1, B2, and C, which are practically all necessary for human nutrition. It is one of the first fruits to be widely grown commercially in numerous countries worldwide [4]. It is mostly consumed either fresh or in prepared meals like ice cream, milk shakes, and preserved fruit juices [5]. It is well renowned for its unique scent, juicy texture, and vivid red color.

To fulfill the need of the local fresh market, processing, and export markets, increasing the area of strawberry farms is one of the key objectives of the agricultural strategy. According to Moradi *et al*. (2011) [6], strawberries are a rich source of natural antioxidants since they have a reasonably high ellagic acid content, which is believed to have anti-carcinogenic properties. One of the few fruit crops that may be cultivated is the strawberry, which produces excellent fruit that usually becomes available for purchase during a period when other fruits are not accessible [7].

Strawberries are grown in all arable parts of the world, from the arctic to the tropics, and are a common dessert crop that millions of people regularly eat. [8]. Strawberry output in over 75 countries is of considerable reportable quantity [9]. Strawberry cultivation may be accomplished using two methods:

runner/nodal segments [10] or runner tips *in vitro* micro propagation reported by Ashrafuzzaman *et al.* 2013. [11] employing various combinations of plant growth regulators. By rooting stolon plants that are created in environments with long days and high temperatures, strawberries are reproduced vegetatively [12]. More than 100 seedlings can be produced from the 10 to 15 stolons that a fully grown strawberry plant can produce [13]. But strawberry farming's ability to reproduce is restricted and challenging due to the heavy human effort and labor needed to get seeds [14]. Furthermore, growing strawberry seedlings using conventional methods might take a considerable amount of time [15]. Kim et al., (2020) also found that new biotechnology techniques have led to significant advancements in the plant breeding industry, and these ground-breaking discoveries are happening all the time [16]. As an alternative to standard propagation methods, mass multiplication *in vitro* using tissue culture has been shown to produce large yields of disease-free plant material studied by Mohan *et al*., 2005 [17]. Hasan *et al*. (2010) also reported that this method works well [18]. However, the rate of multiplication through this typical method is very sluggish, and the viral infections can often be spread by runners during the vegetative propagation phase [19].

Strawberries are prone to viral and mycoplasma infections, much like any other plant that is grown vegetatively [20]. Every year, strawberries require a massive amount of healthy, virus-free planting material, which is challenging to provide through field production of runners. In order to get around this difficulty, commercial strawberry replication uses micropropagation, which allows for the production of many healthy, virus-free plants from tiny explant tissues [21]. In strawberry, single meristems [22], node culture [23], runner tips, and shoot tips have all resulted in successful plant regeneration [24]. Therefore, the goal of the most recent study was to develop a method for the quick clonal multiplication of disease-free strawberry plants in order to guarantee a plentiful supply for commercial production.

Scientific Classification of Strawberry

Kingdom: Plantae
Clade: Tracheophytes
Clade: Angiosperms
Clade: Eudicots
Clade: Rosids
Order: Rosales
Family: Rosaceae
Genus: *Fragaria*
Species: *F.* × *ananassa*
Binomial name: *Fragaria* × *ananassa*

MATERIALS AND METHODS

Explant Collection and Preparation

This research experiment was conducted at the Plant Tissue Culture Laboratory in the campus of Department of Biotechnology Research, Kyaukse Township, Mandalay Region, Myanmar. In order to facilitate future investigations, strawberry plants were obtained from a strawberry grower in Pyin Oo Lwin City, Mandalay Region, Myanmar, and maintained in our lab's plant growth chamber.

Disinfection of the Explants

As explant materials, fresh nodal segments were employed to initiate the culture. Using a sterile surgical blade, we removed around 5 cm nodal segments transplants from the strawberry field and placed them right into distilled water to prevent contamination and cell damage. The collected explants were first thoroughly washed by running tap water for 30 minutes. Subsequently, the explants were cleaned for approximately two minutes with a light detergent and then repeatedly cleansed with distilled water four or five times. Following washing, the explants were cut to a size of 1.0 to 1.5 cm by cutting away extraneous tissue with a surgical blade. After two hours of immersion in a fungicide solution (10 ml/L Carben 50), the removed explants were washed three to four times with sterilized water. They were then immersed in a 500 mg/l Ciprofloxacin tablet for an hour, and after that, they were repeatedly cleaned with sterilized water. After these steps, the explants were brought into laminar flow clean bench and they were subjected to the remaining sterilants such as 20% Cocorex (sodium hypochloride solution) for 10 mins, 70% ethanol for 5 mins and 0.1% mercuric chloride ($HgCl_2$) for 5 mins respectively. After each step, the explants were thoroughly washed with sterilized water for 3-4 times.

Culture Medium and Cultural Condition

The media was created in the laboratory using the ingredients listed in Murashige and Skoog's 1962 publication, MS medium, which was chosen as the ideal culture medium and contained (0.1%) citric acid [25].

Once the MS basal medium had solidified with 0.7% (w/v) agar, it was employed. It contained mineral salts, vitamins, and 3% (w/v) sucrose. Before the pH of the MS media was adjusted to 5.8 (with 0.1 N NaOH and 0.1 N HCl), different amounts of plant growth regulator (PGR), such as 6-benzylaminopurine (BAP), were applied at varying concentrations such as (0, 0.5, 1, 1.5, 2, 2.5 and 3 mg/L). For 15 minutes, media were autoclaved at 121°C and 1.06 kg cm-2. Laboratory-grade chemical supplies were the source of all the MS basal salts, agar, vitamins, and PGRs. All growth phases of the culture bottles were artificially conditioned at 25 ± 2°C, about 60%-70% relative humidity, and with a constant light source (white fluorescent tubes) at a photo-synthetic photon flux density of 30 µmol m-2.

Shoot Multiplication

Fresh strawberry cultivars from local grower in Pyin Oo Lwin City were chosen for the study, and MS medium was added with seven treatments, each of which had varying quantities of BAP: 1) 0 mgL-1 BAP 2) 0.5 BAP mgL-1, 3) 1 mgL-1 BAP, 4) 1.5 mgL-1 BAP, 5) 2 mgL-1 BAP, 6) 2.5 mgL-1 BAP, and 7) 3 mgL-1 BAP. Five replications of the experiment were set up using a completely randomized design (CRD). Each replication had a total of seven treatment concentrations. The proliferation of shoots and the emergence of roots occurred in the culture observations. In order to maintain the proliferation experiments, they were subcultured in a fresh culture medium every four weeks [26]. At the conclusion of the fifth subculture was assessed, and after four months of cultivation, rooting investigations were analyzed.

Rooting and acclimatization

Plants that are fully developed have roots generated from the branches that have been grown *in vitro* [27]. In the shoot regeneration medium with varying BAP concentrations, the majority of the shoots were also rooted. In several instances, multiple roots emerged on their own during *in vitro* shoot regeneration [28,29]. We also discovered in our analysis that the aforementioned rooted development followed the similar path. Since the plantlets were completely grown and matured, we did not employ any auxin hormone to encourage roots growth in our experiment and instead, we transferred them immediately to the acclimatization process. After getting rid of the culture media from the roots, rooted plantlets have been pulled from culture containers and carefully cleaned with tap water. The *in vitro*-grown regenerated plants were placed in tiny polybags filled with previously prepared garden soil, sand, and compost (1:1:1) [30]. After the agar that had been attached to the roots was removed and treated with antifugal solution at a rate of 0.1%, the transfer was completed. Filtered water was used to irrigate the plants. The regenerated plants were transferred into polybags, closed to determine evapotranspiration, and covered with clear plastic after that. After being continuously monitored for 30 days in plant growth chambers, the polybags were taken out of the clear plastic bags and placed in a shaded net house, where they remained for another 7 days. During the month-long transplanting period, these polybags were covered with clear plastic bags with a few holes in them and frequently watered to maintain high humidity in a plant growth chamber setting with day temperatures of 24ºC, night temperatures of 21ºC, relative humidity of 75%, and a photoperiod of 16/8 h [31]. A shade net house was used to outplant hardened plantlets. The plantlets were immediately watered and regular irrigation was required to maintain sufficient soil moisture [32].

Statistical Analysis

In this context, all the shoot micropropagation media were evaluated. In shooting studies, number of shoots, the number of leaves and shoot length (cm) were evaluated. There was no recognized data recorded for rooting. A fully randomized design was used to duplicate each treatment three times for the *in vitro* experiment. It was recorded how many additional shoots sprouted from each original shoot. Also noted was the quantity of leaves that sprouted throughout the experiment. Using the statistical program Minitab 13.1, one-way ANOVA was used to analyze the collected data and P=0.05 was used to measure deviations between means [33]. To evaluate the treatment means, least significant differences (LSD) were computed at the 5% level of significance.

RESULTS AND DISCUSSIONS

The study examined the impact of varying BAP concentrations on the development of shoots from nodal segments containing axillary buds. In an initial trial, numerous shoots were produced by inoculating axillary buds from growing Fragaria plants on MS media with varying dosages of BAP growth regulators. Previous studies on *in vitro* propagation have demonstrated that BAP is more effective than other types of cytokines [34,35]. It is therefore important to figure out the ideal concentration and combination of BAP [36]. According to investigations conducted by Adel and Sawy (2007) [37], Biswas et al. (2007) [38], Sakila et al. (2007) [39], and Harker et al. (2000) [40], BAP plays an important role for strawberry regeneration. These studies highlight the significant role of BAP for strawberry regeneration. Excessive dosages of BAP have been frequently employed for *in vitro* stock culture in several plant species [41-44]. Regarding the quantity of shoots/explants, shoot length/explants and explant regeneration, varying doses of growth regulators exhibited varying responses (Table 1). This table illustrated how BAP affected shoot proliferation following a 30-day culture incubation period. The concentration of 1.5 mg/L BAP produced the greatest number of shoots, although it was not much more than that of other treatments, including control. The maximum length of 1.4 cm was obtained in BAP 2 mg/L, which had the optimal concentration for shoot elongation. In terms of shoot length parameter, it was discovered that this concentration differed considerably from BAP values of 0.5 mg/L and 1.5 mg/L. The best BAP concentrations were likewise discovered at 2 mg/L, which produced 3.667 leaves/explant and differed significantly from the control and 3 mg/L BAP concentrations in terms of the number of leaves produced.

The table 2 presented the effects of BAP on shoot proliferation after a 60-day culture incubation period. With the exception of the control treatment, the concentration of 3 mg/L BAP generated the highest number of shoots (3.600 ± 0.894^{a}), but it did not vary substantially from other treatments such as 0.5 mg/L, 2.5 mg/L, 1 mg/L, 1.5 mg/L, and 2 mg/L. In BAP 1.5 mg/L, which possessed the ideal concentration for shoot elongation for 60-day culture, the maximum length of 1.67 cm was achieved. It was shown that this concentration varied significantly from BAP values of 0 mg/L and 0.5 mg/L in terms of the shoot length parameter. The optimal BAP concentrations were also found to be 3 mg/L, which resulted in 14 leaves per explant and was considerably different from the control and 0.5 mg/L.

After a 90-day culture incubation period, the effects of BAP on shoot proliferation were discovered in Table 3. The concentration of 3 mg/L BAP produced the greatest number of shoots per explant (13.20 ± 4.44^{a}), differentiating it substantially from all other treatments, including the control treatment. The control group had the fewest shoots per explant (3.200 ± 1.304^{c}), which was notably different from all other treatment groups. The maximum length of 3.04 cm was attained in BAP 0.5 mg/L, which was the optimal concentration for shoot elongation for 90 days following culture. It was demonstrated that, in terms of the shoot length, this concentration differed considerably from BAP values of 1 mg/L, 2 mg/L, and 2.5 mg/L. In terms of leaf are counted, it was also discovered that the most suitable BAP concentration was 1 mg/L, producing (24.40 ± 6.88^{a}) leaves per explant. This was significantly lower than the control and BAP concentration of 2 mg/L.

Table 1: Influence of different BAP concentrations on strawberry shoot growth 30-day after culture

BAP (mg/L)	Number of shoots/explants	Shoot length (cm)	Number of leaves/explants
0	1.600 ± 0.548^{c}	1.183 ± 0.426^{ab}	2.333 ± 1.033^{b}
0.5	1.800 ± 0.837^{bc}	0.983 ± 0.293^{b}	3.000 ± 1.265^{ab}
1	2.000 ± 0.707^{abc}	1.233 ± 0.216^{ab}	3.500 ± 1.049^{ab}
1.5	2.600 ± 0.548^{a}	1.000 ± 0.356^{b}	3.333 ± 1.033^{ab}
2	2.400 ± 0.548^{ab}	1.400 ± 0.369^{a}	3.667 ± 0.816^{a}
2.5	2.400 ± 0.548^{ab}	1.217 ± 0.319^{ab}	3.500 ± 1.517^{ab}
3	2.200 ± 0.447^{abc}	1.250 ± 0.327^{ab}	2.833 ± 0.753^{b}

Grouping Information Using the Fisher LSD Method and 95% Confidence Means that do not share a letter are significantly different.

Table 2: Influence of different BAP concentrations on strawberry shoot growth 60-day after culture

BAP (mg/L)	number of shoots/explants	shoot length (cm)	number of leaves/explants
0	1.400 ± 0.548^{b}	1.180 ± 0.449^{c}	7.000 ± 0.707^{d}
0.5	2.800 ± 1.789^{a}	1.240 ± 0.378^{bc}	8.00 ± 3.74^{cd}
1	2.400 ± 0.548^{ab}	1.340 ± 0.321^{abc}	12.20 ± 4.02^{ab}
1.5	2.600 ± 0.548^{ab}	1.660 ± 0.365^{a}	11.800 ± 1.789^{ab}
2	2.400 ± 0.548^{ab}	1.400 ± 0.367^{ab}	8.800 ± 1.924^{bcd}
2.5	3.000 ± 1.225^{a}	1.620 ± 0.277^{ab}	11.00 ± 3.16^{abc}
3	3.600 ± 0.894^{a}	1.480 ± 0.239^{abc}	14.00 ± 2.92^{a}

Grouping Information Using the Fisher LSD Method and 95% Confidence Means that do not share a letter are significantly different.

Table 3: Influence of different BAP concentrations on strawberry shoot growth 90-day after culture

BAP (mg/L)	number of shoots/explants	shoot length (cm)	number of leaves/explants
0	3.200 ± 1.304 [c]	2.540 ± 0.760[ab]	15.40 ± 5.59[bc]
0.5	9.00 ± 3.24[b]	3.040 ± 0.986 [a]	23.60 ± 5.50[a]
1	7.20 ± 2.77[b]	2.200 ± 0.453[b]	24.40 ± 6.88[a]
1.5	10.40 ± 3.29[b]	2.680 ± 0.487[ab]	22.00 ± 6.36[ab]
2	7.000 ± 1.225[b]	2.160 ± 0.416[b]	14.60 ± 2.41[c]
2.5	8.00 ± 2.55[b]	2.180 ± 0.363[b]	18.80 ± 5.63[abc]
3	13.20 ± 4.44[a]	2.260 ± 0.555[ab]	22.00 ± 4.47[ab]

Grouping Information Using the Fisher LSD Method and 95% Confidence Means that do not share a letter are significantly different.

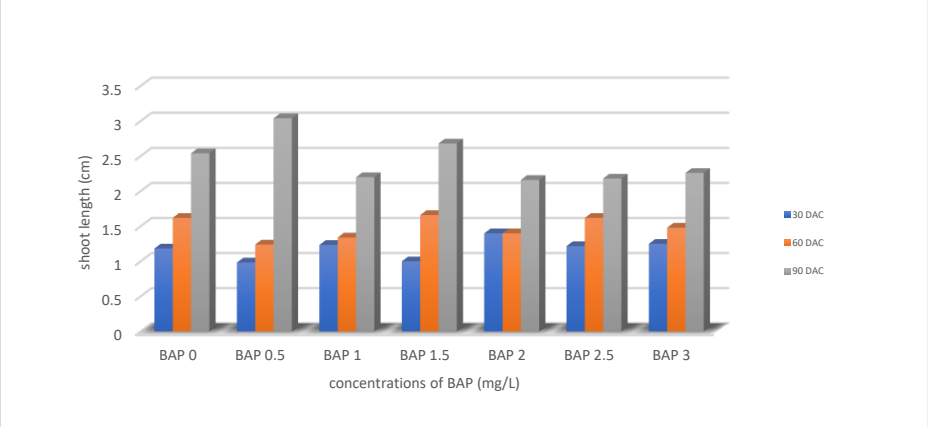

Figure 1: Effect of different BAP concentrations on the length of shoot after 30, 60 and 90 days of culture incubation

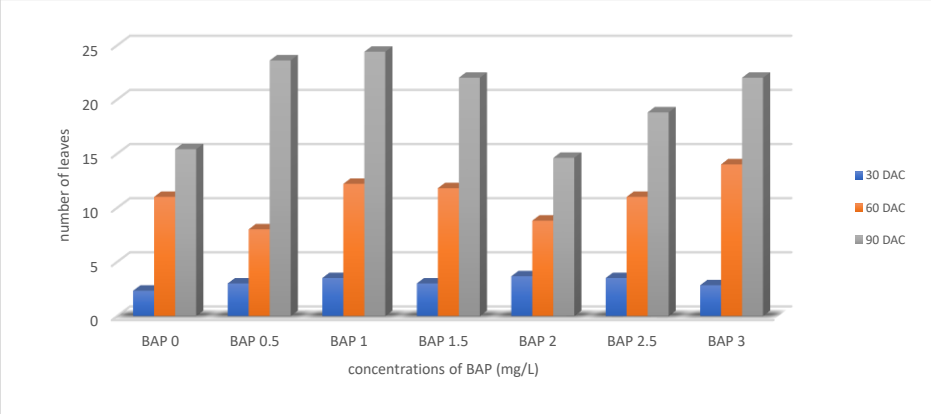

Figure 2: Effect of different BAP concentrations on the number of leaves after 30, 60 and 90 days of culture incubation

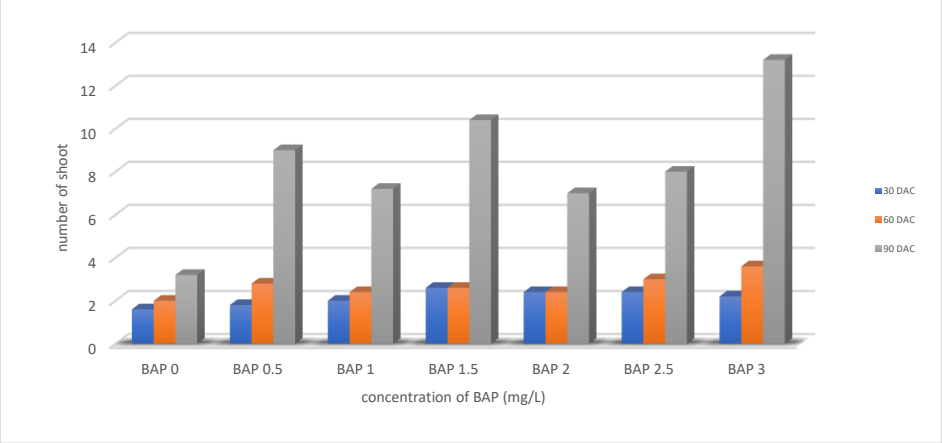

Figure 3: Effect of different BAP concentrations on the number of shoot after 30, 60 and 90 days of culture incubation

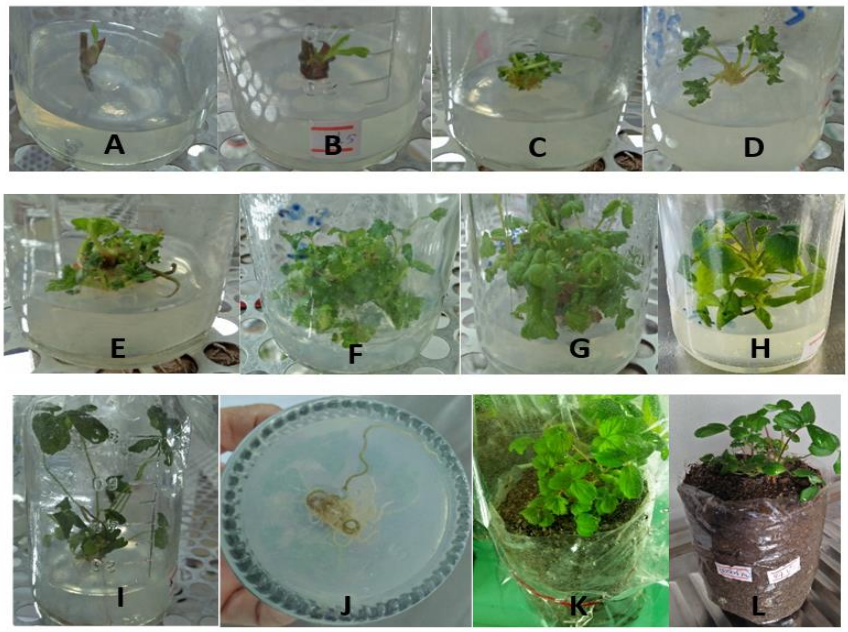

Figure 4: Micropropagation of Strawberry (*Fragaria x ananassa*) (A) Nodal segment as explant (B) Shoot initiation after 2 weeks of culture (C) Shoot development after 3 weeks of culture (D) Leaf proliferation after 4 weeks of culture (E) Shoot multiplication after 5 weeks of culture (F) Shoot proliferation after 8 weeks of culture (G) Leaf maturation after 9 weeks of culture (H) Shoot elongation after 10 weeks of culture (I) Root initiation after 11 weeks of culture (J) Root proliferation after 12 weeks of culture (K) Primary hardening after 2 weeks of transplanting (L) Secondary hardening after 4 weeks of transplanting.

CONCLUSION

The main area of application for plant biotechnology is plant tissue culture [45]. It provides the recovery of high producing types that are resistant to stress, salt, and disease. However, in order to clarify strawberry (*Fragaria x ananassa*) *in vitro* propagation and identify the optimal medium compositions for the greatest response, growth regulator standardization is necessary to ensure maximal germination and shoot proliferation *in vitro* from nodal segments [46]. Nodal explants from mature *Fragaria x ananassa* plants were inoculated on MS medium with varying concentrations of growth regulators of BAP at seven different concentrations (0, 0.5, 1, 1.5, 2, 2.5, and 3 mg/L) in order to optimize the medium for regeneration of strawberry in a preliminary experiment. 3 mg/L BAP was the concentration at which the greatest number of usable shoots was seen. (Figure 1) The concentration of 1.5 mg/L BAP was found to produce the longest shoots. (Figure 2) However, the medium supplemented with 1 mg/L of BAP showed the highest leaf development. (Figure 3) The greatest response in shoot regeneration was therefore shown by higher BAP concentrations, whereas longer shoot length and more leaf development were shown by lower BAP concentrations after 90 days of culture period. The results of this study revealed that strawberry cultivars from nodal segments have the potential to be successfully replicated year-round in order to satisfy the need for high-quality planting material in strawberry-growing regions.

Acknowledgment

This work was supported by the Department of Biotechnology Research (Kyaukse), Ministry of Science and Technology, Myanmar. Furthermore, acknowledged by the author is Dr. Aye Aye Khai, Deputy Director General, Department of Biotechnology study, for her permission and assistance throughout this research effort. The author is also willing to thanks her lab technicians, lab workers and her colleagues for their generosity and kind assistance during this research.

Conflict of interest

There is no conflict of interest.

Financial Support

None declared.

REFERENCES

1. Bhat RP, Devi KM, Jayalaxmi H, Sophia I, Prajna PS. Effect of plant growth regulators on establishment and growth of strawberry (Fragaria x ananassa Duch.) var. chandler in vitro. Agriculture Science Research Journals. 2012;2(12):623-32.
2. Boxus P. Commercial production of strawberry plants produced by meristem culture and micro-propagation, Colloques Scientifiques, Horticultural Abstracts 1983;53: 7669.
3. Gautam H, Kaur R, Sharma DR, Thakur N. A comparative study on in vitro and ex vitro rooting of micropropagated shoots of strawberry (Fragaria x ananassa Duch.). Plant Cell Biotechnol. Mol. Biol. 2001;2(3/4):149- 52.
4. Rattanpal HS, Gill MIS, Sangwan AK. Micropropagation of strawberry through meristem culture. Acta Hort. 2011;890: 149-54.
5. Zebrowska JI, Czernas J, Jawronski J, Hortanski JA. Suitability of strawberry (Fragaria x annassa Duch.) microplants to the field cultivation. Food, Agriculture and Environment. 2003;1: 190-93.
6. Moradi K, Otroshy M, Azimi MR. Micropropagation of strawberry by multiple shoots regeneration tissue cultures. Journal of Agricultural Technology. 2011;7(6):1755-63.
7. Dijkstra J. Research on strawberries focusses on healthy plant material. Expensive cultural method requires excellent material. Fruitteelt-Den-Hang. 1993;83(34):14-15.
8. Adak N, Kaynak L, Pekmezci M, Gubbuk H. The effect of various hormone types on in vitro propagation of strawberry. Acta Hort. 2009;829:305-308.
9. Debnath SC. Strawberry sepal: another explant for thidiazuron-induced adventitious shoot regeneration. In Vitro Cellular & Developmental Biology - Plant. 2005;41:671-76.
10. Jhajhra S, Dashora LK, Singh J, Bhatnagar P, Kumar A, Arya CK. In- vitro Propagation of Strawberry (Fragaria × ananassa Duch.) International Journal of Current Microbiology and Applied Sciences. 2018; 7(10): 3030-3035.
11. Ashrafuzzaman M, Faisal SM, Yadav D, Khanam D, Raihan F. Micropropagation of strawberry (Fragaria ananassa) through runner culture. Bangladesh J. Agril. Res. 2013; 38(3):467-72.
12. Dhukate MR, Kher MM, Vadawale AV, Giri P. Protocol for micropropagation of strawberry (Fragaria × ananassa) cv.

'Sweet Charlie' and 'Winter Dawn'. Environmental and Experimental Biology. 2021;19:1-6.
13. Jan A, Bhat KM, Bhat SJA, Mir MA, Bhat MA, Imtiyaz A. Surface sterilization method for reducing microbial contamination of field grown strawberry explants intended for in vitro culture. African Journal of Biotechnology. 2013; 12(39):5749-53.
14. Madumali HKC, Abeythilakarathna PD, Seran TH. Rooting performance of in vitro microshoots of strawberry (Fragaria x ananassa Duch.) as influenced by plant growth regulators. Agrieast. 2021;15(2):69-73.
15. Neri JC, Meléndez Mori JB, Tejada Alvarado JJ,Vilca Valqui NC, Huaman Huaman E, Oliva M, Goñas M. An Optimized Protocol for Micropropagation and Acclimatization of Strawberry (Fragaria × ananassa Duch.) Variety 'Aroma'. Agronomy. 2022;12:968.
16. Kim HJ, Choi MJ, Lee JN, Suh JT, Kim KD, Kim YH, Nam JH. Occurrence and identification of genetic variation and variation continuity in strawberry tissue culture caused by benzyladenine treatment. Journal of Plant Biotechnology. 2020;47(1):46-52.
17. Mohan R, Chui EA, Biasi LA, Soccol CR. Alternative in vitro propagation: use of sugarcane bagasse as a low-cost support material during rooting stage of strawberry cv. Dover. Braz. Archives Bio. Techn. 2005;48:37-42.
18. Hasan MN, Nigar S, Rabbi MAK, Mizan SB, Rahman MS. Micropropagation of strawberry (Fragaria × ananassa Duch.). International Journal of Sustainable Crop Production. 2010;5(4):36-41.
19. Kosar M, Mahmoud O. Regeneration and histological of plants derived from leaf explants in vitro culture of strawberry. International Journal of Agriculture and Crop Sciences. 2013;5(9):943-50.
20. Zakaria H, Hussein GM, Abdel-Hadi A, Abdel-Hadi, Abdallah NA. Improved regeneration and transformation protocols for three strawberry cultivars. GM Crops & Food: Biotechnology in Agriculture and the Food Chain. 2014;5(1):27-35.
21. Dogan S, Sahin M, Kaya O. Researches on the Reproduction of "Ottoman Strawberry" with Tissue Culture. Alinteri Journal of Agriculture Sciences. 2021; 36(1):27-32.
22. Boxus P. Micropropagation of strawberry via axillary shoot proliferation. In: Plant Cell Culture Protocols. Methods in Molecular Biology. Part III. Plant Propagation In Vitro. Hall RD (ed.) Humana Press Inc. Totowa NJ. 1999;111:103-114.
23. Bhatt ID, Dhar U. Micropropagation of Indian wild strawberry. Plant Cell Tissue Organ Culture. 2000;60:83–88.
24. Litwinczuk W, Okototkiewicz E, Matyaszek I. Development of in vitro shoot cultures of strawberry (Fragaria ananassa Duch.), Senga Sengana, and Elsanta, under the influence of high doses of gibberellic acid. Folia Horticulture Turae. 2009;21(2):43-52.
25. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Journal of Plant Physiology. 1962;15:473-97.
26. Kaundal P, Sharma DR, Kaushal K, Nath AK. Studies on somaclonal variation in strawberry (Fragaria x ananassa Duch.) cultivars. In VII International Symposium on Temperate Zone Fruits in the Tropics and Subtropics. 2003;662:269-75.
27. Hannum SM. Potential impact of strawberries on human health: a review of the science. Crit. Rev. Food Sci. Nutr. 2004; 44:1-17.
28. Passey BJ, Barrett KJ,James DJ. Adventitious shoot regeneration from seven commercial strawberry cultivars (Fragaria × ananasse Duch.) using a range of explant types. Plant Cell Reports. 2003;21:397-401.
29. Sood N, Srivastava R, Singh OS, Gosal SS. Enhancing micropropagation efficiency of strawberry using bandage in liquid media. Jour. Appl. Hort. 2000;2(2):92-93.
30. Mereti M, Grigoriadou K, Levantakis N, Nanos GD. In vitro rooting of Strawberry tree (Arbutus unedo l.) In medium solidified by peat- perlite mixture in combination with agar. Acta Hort. 2003;616:207-210.
31. Negi M, Singh CP, Srivastava RK. In vitro multiplication of Strawberry cv. Chandler. Pantnagar Journal of Research. 2008; 6(2):247-50.
32. Danial GH, Ibrahim DA, Omer MS. Response of running shoot tips of strawberry (Fragaria x ananasa) for in vitro Propagation in Kurdistan Region of Iraq. Int J Environ. Agric. Biotech. 2016;1(2):164-69.
33. Dwivedi SK, Kareem A. Introduction and evaluation of strawberry cultivars in cold arid conditions of Ladakh. Prog. Hort. 2004;36(20):207-210.
34. Arab MM, Yadollahi A, Shojaeiyan A, Shokri S, and Ghojah SM. Effects of nutrient media, different cytokinin types and their concentrations on in vitro multiplication of G× N15 (hybrid of almond×peach) vegetative rootstock. Journal of genetic engineering and biotechnology. 2014;12(2):81-87.
35. Haddadi F, Aziz MA, Saleh G, Rashid AA, Ka- maladini H. Micropropagation of strawberry cv. Camarosa: Prolific shoot regeneration from in vitro shoot tips using thidiazuron with N6-benzylamino-purine. Hort. Science. 2010;45(3):453-456.
36. Weifeng Y, Wanmei J, Shupin Y. Effect of plant growth regulators on adventitious shoot regeneration from strawberry (Fragaria ananassa) leaf discs. Plant Physiol. Commun. 2004; 40(4):447-49.
37. Adel EL, Sawy M. Somaclonal Variation in Micro-propagated Strawberry Detected at the Molecular Level International Journal of Agriculture & Biology. 2007;9:721-25.
38. Biswas MK, Hossain M, Ahmed MB, Roy UK, Karim R, Razvy MA, et al. Multiple Shoots Regeneration of Strawberry under Various Colour Illuminations. American-Eurasian Journal of Scientific Research. 2007;2:133-35.
39. Sakila S, Ahmed MB, Roy UK, Biswas MK, Karim R, Razvy MA, Hossain M, Islam R, Hoque A. Micropropagation of Strawberry (Fragaria × ananassa Duch.) A newly introduced crop in Bangladesh. American-Eurasian Journal of Scientific Research. 2007;2(2):151-54.
40. Harker FR, Elgar HJ, Watkins CB, Jakson PJ, Hallett IC. Physical and mechanical changes in strawberry fruit after high carbon dioxide treatments. Postharvest Biology and Technology. 2000;19:139-46.
41. Meyers K, Watkins CB, Pritts MP, Liu RH. Antioxidant and antiproliferative activities of strawberries. Journal of Agricultural and Food Chemistry. 2003;51:6887-92.
42. Diengngan S, Murthy NS. Influence of plant growth promoting substances in migropropagation of strawberry cv. Festival. The Bioscan. 2014;9(4):1491-93.
43. Emarah. Factors affecting propagation of strawberry (Fragaria spp.) through tissue cultures. J. Product. & Dev. 2008;13 (1):191-12.
44. Youssief EY. In vitro propagation of strawberry (Fragaria x annanasa Duch.) through organogenesis via runner tips. M.Sc. dissertation in Biology, University of Gaza, Faculty of Science, Palestine. 2009.
45. Singh AK, Pandey SN. Genotypic variation among strawberry cultivars for shoot organogenesis. Acta Horticulturae. 2004;662:277-80.
46. Rahman MW, Zohora S, Talukder MAI, Kayess MO. Effect of different hormone combinations on callus induction and plant regeneration of strawberry. International Journal of Advanced Research. 2015;3(6):1244-50.