International Journal of New Developments in Engineering and Society
ISSN 2522-3488 Vol. 4, Issue 1: 52-60, DOI: 10.25236/IJNDES.040108

Optimization of The Rapid Propagation System for Strawberry Tissue Culture Seedlings through Temporary Immersion Bioreactors (Tibs)

Mengxing Lv, Jing Lv, Xihan Zhang, Liting Qiu, Jianzhou Ma, Yanan Ruan, Song Jin, Jiani Liu, Zebin Chen*

School of Agronomy and Life Sciences, Kunming University, Kunming, Yunnan Province, China
**Corresponding Author*

ABSTRACT. *This paper aims to optimize the rapid propagation system of strawberry tissue culture seedlings based on the intermittent submerged bioreactor (TIBs). The tissue culture plantlet of "Benihoppe" after primary culture was used as the explant and the TIBs system was used for rapid propagation, so as to study the effects of different generations, inoculation densities, intermittent immersion frequencies and immersion time on the proliferation of strawberry tissue culture seedlings. The results showed that the multiplication times of the third, fourth, fifth and sixth generations were higher, and multiplication rate of the fifth generation was the highest with 70.0 times. When the inoculation density was more than 4 strains / bottle, the pollution rate was 15.8%; when the inoculation density was less than 3 strains / bottle, there was no pollution. When the frequency of intermittent immersion was more than 5 min / 3 h, the pollution rate of tissue culture seedlings increased with the increase of intermittent immersion frequency. When the frequency of intermittent immersion reached 5 min / 4 h, the pollution rate was the highest, and the growth potential of tissue culture seedlings was also poor. The highest multiplication was 71.0 times after 25 minutes immersion every 1 hour. Therefore, good culture effect can be obtained by selecting the 5th generation of subculture plantlets, adopts the inoculation density of 2 plants / bottle and the intermittent immersion frequency of 10 min / 1 h, and uses the hormone combination of 3.0 mg / L 6 - BA + 0.01 mg / L NAA.*

KEYWORDS: *Strawberry, Immersion, Bioreactor, Tissue culture seedling, Rapid propagation*

1. Introduction

Strawberry "Benihoppe" is an early maturing variety bred by the hybridization of "Akihim" and "Sachinoka". It is popular among consumers because of its large fruit size and sweet flesh. The variety has strong continuous fruit bearing, vigorous

ISSN 2522-3488 Vol. 4, Issue 1: 52-60, DOI: 10.25236/IJNDES.040108

growth and good yield. The average yield per plant is more than 350 g, and the yield per 6676.7 m^2 is about 2800 kg; it is the main variety recommended by the 7th World Strawberry Exhibition [1]. At present, most Chinese researchers study the plant tissue culture through the traditional solid / semi-solid culture method, which limits the application of high-efficiency automatic control technology, resulting in the slow promotion of high-quality seedlings. The intermittent submerged bioreactor (TIBs) is a system for the large-scale cultivation of plant tissues. Its principle is to use liquid culture media and filtered air pressure as the driving force to carry out intermittent cultivation of plant tissue culture seedlings to obtain the maximum proliferation rate [2]. The system is highly automatic. In the process of training, materials do not need to be transferred, which saves manpower and material resources and greatly reduces production costs. To explore the application effect of TIBs in tissue culture of strawberry "Benihoppe" (Fragaria × ananassa Duch. "Benihoppe") can provide technical support for improving the propagation efficiency of strawberry tissue culture seedlings and its industrial production. Qiaoli Zhang and colleagues [1] used the third generation seedlings of " Benihoppe" induced by virus-free treatment of stem tip as experimental materials, and used the intermittent submerged bioreactor to study the proliferation technology of tissue culture seedlings. It was found that the proliferation rate of each tissue culture seedling of strawberry using the TIBs system was about 3 times of that of traditional culture method. Meiping Gao and coworkers [3] used the tissue culture seedlings of Guiti No.2 Eleocharis tuberosa tissue culture seedlings obtained from the initial induction culture as explants, and conducted tissue culture fast propagation through the TIBs system to study the effects of different generations, inoculation densities, hormone combinations and intermittent immersion frequencies of tissue culture seedlings on the proliferation of tissue culture. The results showed that to achieve better culture results, in the TIBs system, the subculture plantlets of the 5th generation of Eleocharis tuberosa should be selected; the MS + 3.0 mg / L 6-BA + 0.01 mg / L NAA + 30.0 g / L sucrose with pH 6.0 could be used as the proliferation medium, with the inoculation density of 10 clumps / bottle and the intermittent immersion frequency of reactor 10 min / 6 h. Weiqing Dong and his team [4] used the adventitious buds obtained from the virus-free induction of meristem of Guitaro No.2, a new variety of Lipu Taro, as explants, and selected the best hormone combination suitable for the proliferation and rooting of tissue culture seedlings by the TIBs culture system. they analyzed the effects of different subculture materials, inoculation densities and frequencies of immersion interval on the proliferation and rooting of tissue culture seedlings. The results showed that the TIBs culture system could effectively improve the proliferation of tissue culture plantlets of Lipu Taro. The proliferation rate was 28.96 times, about 10 times of the traditional solid culture method (2.87 times), and the plant height and rooting number of tissue culture plantlets were significantly higher than those of the traditional solid cultured plantlets. Meiping Gao and other scholars [5] used the temporary immersion bioreactor system (TIBs) and tissue culture plantlets induced by the stem tip of Sagittifolia sagittifolia as materials to carry out the research on tissue culture and rapid propagation technology, such as the selection of hormone combination for the rapid propagation of Sagittaria tissue culture, the culture effect

ISSN 2522-3488 Vol. 4, Issue 1: 52-60, DOI: 10.25236/IJNDES.040108

of different generations of Sagittaria subculture materials, the influence of different inoculation densities on the proliferation of Sagittaria tissue culture and the influence of different intermittent frequencies on the rapid propagation of Sagittaria tissue culture. The results showed that the TIBs system could make the first generation of Sagittifolia sagittifolia tissue culture seedlings proliferate for more than 19.5 times, which is more than 3 times higher than the traditional method. At present, there are few reports on the optimization of strawberry tissue culture system in the temporary immersion bioreactors system (TIBs). In this study, strawberry "Benihoppe" tissue culture seedlings obtained from the primary culture were used as explants, and the TIBs system was used for tissue culture and rapid propagation, in order to study the effects of different sub-generations, inoculation densities, intermittent immersion frequencies and different immersion time on the proliferation of strawberry tissue cultured seedlings, and provide technical support for the industrialization and automatic production of strawberry tissue culture seedlings.

2. Research Materials and Methods

2.1 Research Materials

The plantlets of strawberry "Benihoppe" (Fragaria × ananassa Duch. "Benihoppe") provided by the Experimental Centre of Plant Tissue Culture of the School of Agronomy and Life Sciences, Kunming University were used as experimental materials. The propagation medium was MS + 1.0 mg / L BA + 0.05 mg / L NAA + 30.0 g / l sucrose. The TIBs system was established according to the design idea of Lorenz and coworkers [6]. In the TIBs system, both the culture bottle and the storage bottle were 0.5L blue capped glass bottles, with the height of 15cm and the diameter of 7cm. The volume of liquid medium in the storage bottle was 0.25 L / bottle.

2.2 Culture Conditions

The light intensity is 1500 Lx; the culture temperature range is 28 ± 2 ℃, with light for 16 hours per day and dark for 8 hours per day. The proliferation culture lasted for 30 days. Three TIBs systems were set for each treatment and repeated for three times.

2.3 Effects of Different Generations of Tissue Culture Seedlings on the Tissue Culture of Tibs System

Tissue culture seedlings of the 1st to 7th subculture generations were used as experimental materials. The culture medium formula and TIBs culture conditions were the same as 2.1 and 2.2; the pH value was 5.8 - 6.0; the intermittent immersion frequency of TIBs system was 5 min / 1 h; the inoculation density was 2 plants / bottle.

ISSN 2522-3488 Vol. 4, Issue 1: 52-60, DOI: 10.25236/IJNDES.040108

2.4 The Proliferation Effects of Different Inoculation Densities in Tibs System

Tissue culture seedlings of the 5th subculture generation was used as the material. The culture medium formula was the same as 2.1; the culture condition of TIBs was the same as 2.2; the inoculation densities of tissue culture seedlings were set as 1, 2, 3 and 4 plants / bottle respectively.

2.5 Effect of Different Intermittent Immersion Frequencies on Tissue Culture of Tibs System

The fifth generation of sub-cultured plantlets were used as experimental materials. The medium formula was the same as 2.1; the inoculation density was 2 plants / bottle; the intermittent immersion frequencies of TIBs system were set as 5 min / 1 h, 5 min / 2 h, 5 min / 3 h and 5 min / 4 H. Other culture conditions are the same as 2.2.

2.6 The Effect of Different Immersion Time on Tissue Culture of Tibs System

Sub-cultured plantlets of the fifth generation were used as experimental materials. The medium formula was the same as 2.1; the inoculation density was 2 plants / bottle; the time of plantlets immersed in the medium was set as 5, 10, 15, 20 and 25 min / 1 h.

2.7 Effects of Different Hormone Combinations on Tissue Culture in the Tibs System

The fifth generation of sub-cultured plantlets were used as experimental materials. The inoculation density was 2 plants / bottle; the frequency of intermittent immersion was 5 min / 1 h; the concentration gradients of 6-BA were set as 1.0, 1.5, 2.0, 3.0, 4.0 mg / L, and the concentrations of NAA were set as 0.01 and 0.05 mg / L.

2.8 Statistical Analysis

Data were analyzed by software DPS 7.05 through variance analysis.

3. Results and Analysis

3.1 Proliferation Effects of Strawberry Subculture Seedlings of Different Generations in the Tibs System

The first to seventh generations of strawberry plantlets after the primary induction culture were transferred to the TIBs system. After 30 days of culture, the multiplication multiples of the 3rd, 4th, 5th and 6th generations of the sub-cultured

ISSN 2522-3488 Vol. 4, Issue 1: 52-60, DOI: 10.25236/IJNDES.040108

plantlets were higher, as shown in Table 1. The proliferation rate of the 5th generation of sub-cultured plantlets was the highest, which was 70.0 times; the proliferation rate of the 6th generation began to decline, which was 66.3 times; the proliferation rate of the 7th generation decreased to the lowest of 40.1 times. With the increase of subculture generation, the proliferation time of the tissue culture seedling was prolonged. There was no significant difference in the proliferation time of the third, fourth, fifth and sixth generations, all of which began to proliferate at about 17.0 days. Therefore, in order to ensure that the material is sufficient and does not affect the proliferation rate, the fifth generation material is the best for the rapid propagation of strawberry tissue culture seedlings in the TIBs system.

Table 1 Proliferation Effects Of Strawberry Subculture Seedlings of Different Generations in the Tibs System.

Subculture generation	Proliferation time (day)	number of bud proliferation	proliferation rate
1	15.1 d	81.9 d	41.0 d
2	16.4 c	101.6 c	50.8 c
3	17.0 b	130.4 b	65.2 b
4	17.2 b	131.3 b	65.7 b
5	17.6 b	133.1 b	70.0 b
6	17.8 b	132.5 b	66.3 b
7	18 a	80.3 a	40.1 b

Note: Different lowercase letters after the data in the same column respectively indicate the significant difference level ($P < 0.5$); the same as below.

3.2 Proliferation Effects of Different Inoculation Densities in the Tibs System

The 5th generation of tissue culture seedlings was selected to study the effects of different inoculation densities on the rapid propagation of tissue culture of strawberry (Table 2). After 17 days of culture, it was found that with the increase of inoculation density, the number of proliferating buds increased gradually, but the pollution rate also increased. When the inoculation density was more than 4 plants / bottle, the pollution rate reached 15.8%; when the inoculation density was less than 3 plants / bottle, there was no pollution. Therefore, in order to achieve the culture effect of zero pollution and high-efficiency proliferation, the density of 2 plants / bottle was selected for subsequent experimental study.

Table 2 Proliferation Effects Of Different Inoculation Densities in the Tibs System.

inoculation density (plant/bottle)	pollution rate (%)	number of bud proliferation	proliferation rate
1	0 c	32.6 d	32.6 d
2	0 c	130.4 c	65.2 c
3	10.2 b	293.4 b	97.8 b

ISSN 2522-3488 Vol. 4, Issue 1: 52-60, DOI: 10.25236/IJNDES.040108

4	15.8 a	521.6 a	130.4 a

3.3 Proliferation Effects of Different Intermittent Immersion Frequencies in the Tibs System

The effects of intermittent immersion frequencies on the rapid propagation of strawberry tissue culture were studied by selecting the 5th generation of sub-cultured plantlets and adopting the inoculation density of 2 plants / bottle. When the intermittent immersion frequency was more than 5 min / 3 h (as shown in Table 3), the pollution rate of tissue culture seedlings increased with the increase of intermittent immersion frequency. When the intermittent immersion frequency reached 5 min / 4 h, the pollution rate was the highest, and the growth potential of tissue culture seedlings was also poor. The proliferation rate decreased with the increase of the intermittent immersion frequency. When the intermittent immersion frequencies were 5 min / 1 h and 5 min / 4 h, the proliferation rates of buds were 66.7 times and 59.1 times respectively. It can be seen that short-term intermittent soaking is not conducive to the multiplication and growth of strawberry tissue culture seedlings and cannot control the pollution rate. Therefore, the suitable intermittent immersion frequency is 5 min / 1 h.

Table 3 Proliferation Effects Of Different Intermittent Immersion Frequencies in the Tibs System.

intermittent immersion frequencies	pollution rate (%)	number of bud proliferation	proliferation rate	growth potential
5 min / 1 h	0 c	133.4 a	66.7 a	+++
5 min / 2 h	0 c	130.6 ab	65.3 ab	+++
5 min / 3 h	12.5 b	124.8 b	62.4 b	+++
5 min / 4 h	13.4 a	118.2 c	59.1 c	++

Note: "+" means normal growth of strawberry seedlings; more "+" represents better growth potential.

3.4 Proliferation Effects of Different Immersion Time in the Tibs System

With the increase of immersion time, the proliferation rate of tissue culture seedlings gradually increased (Table 4), among which, the proliferation rate of 25 minutes immersion per hour was the highest, which was 71.0 times. Increasing the immersion time could promote the growth of tissue culture seedlings. When the immersion time was more than 15 minutes, the vitrification rate of tissue culture seedlings was 13.1%. When the immersion time was 25 minutes, the vitrification rate was the highest, 20.2%. In order to control the efficiency of tissue culture seedlings, it is considered that 10 minutes immersion in one hour is beneficial to the normal growth of tissue culture seedlings.

ISSN 2522-3488 Vol. 4, Issue 1: 52-60, DOI: 10.25236/IJNDES.040108

Table 4 Proliferation Effects Of Different Immersion Time in the Tibs System.

immersion time (min / 1 h)	vitrification seedling rate (%)	number of bud proliferation	proliferation rate	growth potential
5	0 d	133.4 e	66.7 e	+++
10	0 d	137.6 d	68.8 d	++++
15	13.1 c	138.6 c	69.3 c	++++
20	17.4 b	140.6 b	70.3 b	++++
25	20.2 a	142.0 a	71.0 a	++++

3.5 Effects of Different Hormone Combinations on the Proliferation of Strawberry Tissue Culture Seedlings

The 5th generation of subculture plantlets, the inoculation density of 2 plants / bottle and the intermittent immersion frequency of 10 min / 1 h of were selected; seedlings were transferred to the proliferation medium containing different concentrations of 6-BA and NAA. The proliferation rate increased gradually with the increase of 6-BA concentration (shown in Table 5). When 0.05 and 0.01 mg / L NAA were added to the medium at 4.0 mg / L 6-BA, the multiplication multiples of buds were the highest, which were 73.0 and 80.2 times respectively. NAA 0.01 mg / l was more conducive to the proliferation of buds than 0.05 mg / L, and the proliferation rate was higher, but the buds cluster was smaller and the growth potential was slightly worse. When the concentration of 6 - BA reached 4.0 mg / L, seedlings were thin and compact. In order to ensure the quality of tissue culture plantlets and increase the multiplication multiple as much as possible, it is considered that 3.0 mg / L 6 - BA + 0.01 mg / L NAA is the best hormone combination, under which the multiplication multiple of tissue culture plantlets can reach 75.6 times.

Table 5 Effects of Different Hormone Combinations on the Proliferation of Strawberry Tissue Culture Seedlings.

combination number	6 - BA (mg / L)	NAA (mg / L)	number of bud proliferation	proliferation rate	growth potential
1	1.0	0.05	112.2 h	56.1 h	+
2	1.5	0.05	125.0 g	62.5 g	+++
3	2.0	0.05	133.6 e	66.8 e	++++
4	3.0	0.05	140.8 de	70.4 de	++++
5	4.0	0.05	146.0 c	73.0 c	+++
6	1.0	0.01	128.2 f	64.1 f	+
7	1.5	0.01	134.4 e	67.2 e	++

ISSN 2522-3488 Vol. 4, Issue 1: 52-60, DOI: 10.25236/IJNDES.040108

8	2.0	0.01	143.2 d	71.6 d	+++
9	3.0	0.01	151.2 b	75.6 b	++++
10	4.0	0.01	160.4 a	80.2 a	+++

4. Conclusion

In this study, the TIBs system was used to carry out tissue culture and rapid propagation, and study the effects of different generations, inoculation densities, intermittent immersion frequencies and immersion time on the proliferation of strawberry tissue culture seedlings. The results 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 / 1 h, and selecting the hormone combination of 3.0 mg / L 6 - BA + 0.01 mg / L NAA. At the same time, we found some factors that were not included in this study would affect the culture effect of TIBs. For instance, container volumes and sugar concentrations of the nutrient solution are also factors affecting the biomass and quality of culture. Therefore, the follow-up study can be further improved this research.

A large number of studies showed that the proliferation rate of tissue culture in the TIBs system was much higher than that in the traditional solid / semi-solid culture. The reason may be related to the good gas exchange environment of the TIBs system. At the same time, the recycling of medium can make full use of nutrient components and effectively prevent nutrient deposition and the accumulation of harmful substances [7]. It has also been reported that the liquid culture medium in TIBs system directly contacts with the tissue, so it can promote the absorption of nutrients and stimulate plant hormones, and increase the number of buds. At the same time, the continuous intermittent immersion vibration can inhibit the apical dominance and lead to more induction and proliferation of axillary buds. In addition, in the TIBs system, oxygen is continuously supplied to plant tissues, resulting in the rapid growth of plant tissues [8]. Using the TIBs system on a large scale to achieve the rapid propagation and production of healthy strawberry seedlings is an ideal choice for the strawberry industry.

Acknowledgement

This paper was supported by the Foundation for Projects of the University Students' Innovation and Entrepreneurship Training Program of Yunnan Province in 2019 (Project No.: S201911393033) and the Foundation for Basic Research Projects of Local Undergraduate Universities and Colleges of Yunnan Province (Project No.: 2017FH001-041, 2017FH001-035, 2019FH001-008).

ISSN 2522-3488 Vol. 4, Issue 1: 52-60, DOI: 10.25236/IJNDES.040108

References

[1] Zhang, Q.L., Shao, W., Gao, L.M., et al.(2014). Parameters of Rapid Propagation of Strawberry Virus-Free Seedlings in Intermittent Submerged Bioreactor. Journal of Beijing University of Agriculture, vol. 29, no. 2, pp. 20-24.

[2] Lian, M.L., Piao, X.C., Wang, Y., et al.(2004). Application of Intermittent Submerged Bioreactor in the Propagation of Chenxingcao Seedlings. Acta Horticulturae Sinica, vol. 31, no. 4, pp. 532-532.

[3] Gao, M.P., Lin, Z.C., Zhang, C., et al.(2016). Efficient Propagation Technology of Water Chestnut Tissue Culture Seedlings in Intermittent Submerged Bioreactor (TIBs). Journal of Southern Agriculture, no. 10, pp. 25-29.

[4] Dong, W.Q., He, F.L., Jiang, W., et al.(2018). Tissue Culture and Rapid Propagation Optimization of Lipu Taro Based on Intermittent Submerged Bioreactor. Journal of Southern Agriculture, vol. 49, no. 6, pp. 1164-1170.

[5] Gao, M.P., Lin, Z.C., Zhang, C., et al.(2016). Study on Tissue Culture and Rapid Propagation of Sagittaria Sagittarius Using Intermittent Submerged Bioreactor. Southwest China Journal of Agricultural Sciences, vol. 29, no. 11, pp. 2704-2708.

[6] José, C.L., Boris, L.G., Escalona, M., et al.(1998). Sugarcane Shoot Formation in an Improved Temporary Immersion System. Plant Cell Tissue & Organ Culture, vol. 54, no. 3, pp. 197-200.

[7] Shi, K., Zheng, C.X., Zhang, Y.T., et al.(2017). Batch Immersion Liquid Culture Technology and its Application in Micro Propagation of Berry Fruit Plants. Plant Physiology Journal, vol. 53, no. 4, pp. 513-520,.

[8] Zhao, W.F., Wang, L.H.(2007). Study on the Application of Intermittent Submerged Bioreactor in Large-Scale Tissue Culture. Journal of Anhui Agricultural Sciences, vol. 35, no. 2, pp. 317-320.