

Rapid Micropropagation of *Sapium sebiferum* using Segments with Axillary Bud for Forest Bioenergy

Youming Xu^{1*}, Ningning Wang², Yawei Xie³

College of Horticulture and Forestry, Huazhong Agricultural University, Wuhan China

*Corresponding Author

Received:- 05 December 2021/ Revised:- 13 December 2021/ Accepted:- 20 December 2021/ Published: 31-12-2021

Copyright © 2021 International Journal of Environmental and Agriculture Research

This is an Open-Access article distributed under the terms of the Creative Commons Attribution

Non-Commercial License (<https://creativecommons.org/licenses/by-nc/4.0>) which permits unrestricted

Non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract— *Sapium sebiferum* is one of the four woody oil tree species in China for bioenergy forest. Its seeds have high oil content and are widely used. It is an excellent tree species that can be cultivated as a renewable energy source. An experiment was conducted to study culture in vitro and rapid propagation of *S. sebiferum* using segments with axillary bud. The results showed that the survival rate could be reached to 50.0% when the explants were disinfected with 70% ethanol for 30 seconds and with 0.1% HgCl₂ for 10 minutes. In induction culture, the lateral buds began to sprout about 5 days later, and after 20 days, the buds gradually differentiated. Among the three basic mediums, WPM medium has the highest effective bud induction rate, the effect of 6-benzyladenine (6-BA) mass concentration on axillary bud induction rate is extremely significant. The optimal medium WPM +6-BA 0.5mg/L+ NAA 0.05mg/L, conducting bud induction, and the induction rate could reach 62.9% after 30 days. Multiplication culture was carried out in the medium MS + 6-BA 1.0 mg/L +NAA 0.5 mg/L+ KT 0.5 mg/L, the proliferation effect was the best, with the multiplication rate of 81.2% and proliferation multiple of 3.2. The results of variance analysis showed that Naphthalene acetic acid (NAA) is the main factor affecting bud proliferation, followed by 6-BA. The rooting rate was up to 93.3% after induction in the medium 1/2 MS+ IBA 0.5mg/L. The establishment of rapid propagation technology system through tissue culture is an important way to solve the shortage of high-quality seedlings and provide technical support for the establishment of large scale *S. sebiferum* energy forest.

Keywords— *Sapium sebiferum*; Bioenergy; Stem section; Culture in vitro; Rapid propagation.

I. INTRODUCTION

Sapium sebiferum (Roxb.) is a deciduous tree of *Sapium* in Euphorbiaceae, also known as wood wax tree. *S. sebiferum* is a drought-tolerant, short-term water-tolerant tree. There is a cultivation history of more than 1400 years in China (Editorial committee of Chinese flora 1980). It is one of the four major woody oil-bearing plants with strong cultivation adaptability, high oil content and wide use. It is an important economic bioenergy tree in China (Li et al. 2011). *S. sebiferum* is an excellent afforestation tree for timber forest and shelter forest in China with fast growth and long life. Especially as a scenic forest, it has a tall crown and different colors in four seasons, which is of great ornamental value. The root bark, bark and leaves of *Sapium sebiferum* are important raw materials of traditional Chinese medicine for the treatment of cirrhosis ascites, eczema and dermatitis. The leaf phenol extract has many effects, such as antioxidant, anti-inflammatory and anti ACD (Fu et al. 2015) (allergic contact dermatitis). The seed is covered with a layer of wax skin, which can be used to extract sapium fat (also known as Chinese (vegetable) tallow); the kernel can be extracted sapium oil (Li and Liu 2011). 100 kg seeds can be extracted 23 to 25 kg of sapium fat and 16 to 18kg of sapium oil. The well-operated sapium forest, the oil yield of seeds is as high as 40%, higher than that of *Camellia oleifera*, and it is also higher than *Elaeis guineensis* Jacq known as "oil king". The oil extracted from the seeds can be used not only as an edible oil source or an industrial oil source, but also as a power oil source, and is an inexhaustible source of renewable oil (Li et al. 2011; Shi and Guo 2001; Tan 2013; Li and Xu 2006; Peng 2013). With the deepening of biodiesel research and the accelerated development of biomass energy forestry industrialization, As a kind of high oil content substance, *S. sebiferum* seeds have a quite considerable prospect to be converted into biodiesel. Biodiesel is a typical "green energy" with the advantages of extremely low sulfur content, low aromatic hydrocarbon content, high oxygen content, high cetane content, and low exhaust gas emission (Wu et al. 2011; Helwani et al. 2009; Chen 2006; Campanelli et al. 2010; Hayyan and Mjalli 2010). The importance of wood biomass energy has been raised many times (Venketeswaran et al. 1983; Bajaj 1986).

Therefore, it is necessary to carry out the research on the breeding, the rapid propagation, cytochemical composition, secondary metabolites, and the comprehensive utilization technology of seed production biodiesel, which is of great significance to alleviate the energy shortage, improve the ecological environment, and promote the adjustment of rural industrial structure. The establishment of *S. sebiferum* energy forest requires a large number of high-quality seedlings. Compared with traditional propagation methods (such as seedling planting or grafting), this method can produce plant materials for breeding or other purposes with less labor and lower cost in a shorter time (Bommineni et al. 2001). The establishment of rapid propagation technology system through tissue culture is an important way to solve the shortage of high quality seedlings. With regard to tissue culture, previous researcher used callus to regenerate the plants in vitro (Venketeswaran and Gandhi 1982), and performed in vitro propagation by means of bud proliferation from nodal explants (Siril and Dhar 1997). In this study, stem segments of *S. sebiferum* were cultured in vitro, and its high-quality seedlings rapid propagation technology system was studied to provide technical support for the establishment of large-scale *S. sebiferum* energy forest.

II. MATERIALS AND METHODS

The test materials were collected from *S. sebiferum* plantation of Huazhong Agricultural University. The semi lignified branches on the healthy, vigorous and disease-free plants with a diameter of 0.2-0.4cm were selected.

Primary culture: The inoculated explants were stem segments with buds. Remove the leaves from the branches indoors and wash them with tap water several times. After soaking in 1% washing powder water for 10 minutes, the surface of the explants was cleaned with a brush, and then washed with water for 2 hours.

In aseptic condition, 70% alcohol was used to disinfect for 30 s, then 0.1% HgCl_2 was used to disinfect at different minutes (5, 8, 10, 12). After 4 rinses with sterile distilled water, the stem segment with axillary bud was cut into 1-2 cm, each stem segment has one or two buds, which was used to select the optimal conditions for disinfection. Each treatment was repeated 3 times, with 16 explants.

Selection of optimal inducement conditions: The basic medium was MS, WPM and B5, and the growth regulators were 6-BA (0.5, 1.0, 2.0) and NAA (0.05, 0.1, 0), 6-BA and NAA are mass concentration (mg/L). The orthogonal experiment was carried out with 3 factors and 3 levels of basic medium. There are 9 treatments in total; each treatment was repeated 3 times, with 30 explants.

Subculture: After 30 days of primary culture, the induced buds were cut and inoculated on subculture medium, observe their proliferation and make statistical analysis. In the subculture, MS was used as the basic medium, and the orthogonal experiment was carried out at the level of 6-BA (0.5, 1.0, 2.0) mass concentration, KT (0.1, 0.5, 1.0) mass concentration and NAA (0.1, 0.5, 0) mass concentration. Each treatment was inoculated with 30 explants, and each treatment was repeated 3 times. There are 9 treatments in total.

Induction rate = (number of induced explants / total number of inoculated explants) $\times$ 100%.

Rooting culture: Inoculate the healthy shoots obtained from Subculture on rooting medium for root induction. The rooting was counted after 30 days. 1 / 2MS were used as the basic medium for rooting induction, and 3 treatments were carried out with different mass concentrations of IBA (0.1, 0.5, 1.0). Each treatment was inoculated with 15 explants, and each treatment was repeated 3 times.

The culture conditions, unless otherwise specified, are $(25 \pm 2)^\circ\text{C}$ (degree centigrade), 14h / d, 1500-2000lx. For each medium, 0.7% agar and 3% sucrose (1.5% for rooting medium) were added. The pH of the medium is 5.8-6.0, the growth regulators units are mg/L.

Data analysis: SAS 9.4 and EXCEL2003 are used for data statistics, analysis of variance and multiple comparisons.

Data availability: The manuscript includes all data that support the findings of the present study.

III. RESULTS AND ANALYSIS

3.1 Effect of different treatment time of 0.1% HgCl_2 on pollution rate of explants.

In aseptic condition, *S. sebiferum* explant was sterilized with 70% alcohol for 30s, and then sterilized with 0.1% HgCl_2 for 5, 8, 10 and 12 minutes, than the pollution rate and survival rate was counted after 2 weeks (Table 1). With the increase of 0.1% mercuric chloride treatment time, the pollution rate decreased, but the germination rate also decreased. This show that the

treatment time of 0.1% HgCl_2 is increased, which is beneficial to reduce pollution; when the treatment time exceeds a certain limit, it will cause serious damage to plant tissue cells, so it should be considered comprehensively according to the survival rate and pollution rate. In terms of *S. sebiferum* explants, 0.1% HgCl_2 treatment for 10 minutes is the best sterilization method, the pollution rate can be reduced to 26.5%, and the survival rate can reach 50.0%.

TABLE 1
EFFECT OF DIFFERENT TREATMENT TIME OF 0.1% HgCl ON POLLUTION RATE OF *S. sebiferum* EXPLANTS

Treatment time/min	Pollution rate%	Browning mortality%	Survival rate%
5	46.8	15.6	37.6
8	28.6	21.4	50.0
10	26.5	23.5	50.0
12	25.8	25.8	48.4

3.2 Effects of different hormone combinations on axillary bud induction of *S. sebiferum*.

The stem segment of *S. sebiferum* was inoculated in the medium, and the lateral buds began to sprout about 5 days later (Fig.1-A). At the same time, a small number of stem base began to expand, and buds appeared. After 15 days, the lateral buds opened, and after 20 days, the buds gradually differentiated (Fig.1-B). The statistical results of the effects of different hormone combinations on the axillary bud induction of *S. sebiferum* are shown in Table 2. The effective bud induction rate of stem segment with axillary bud in medium 4 was significantly higher than that of other treatments, and the induction rate was 62.9%

The axillary buds can be activated as early as 3 days. Medium 1 and medium 5 were also effective, the induction rates were 44.4% and 45.7% respectively. Treatment 9 was significantly lower than other treatments, only 28.6%, and could be activated in 5-7 days.

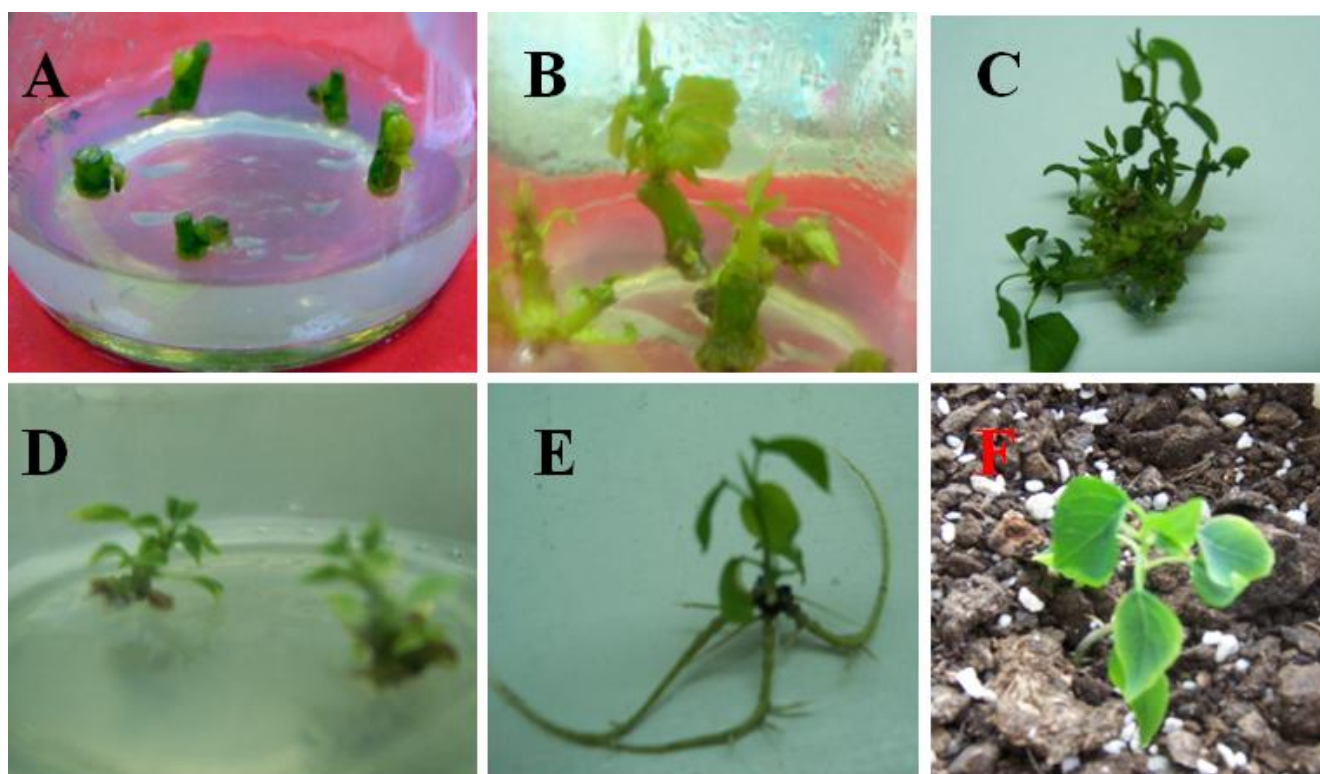


FIGURE 1: Growth of *S. sebiferum* in different stages of tissue culture. A: the buds are beginning to sprout after 5 days. B: The axillary buds grew well after 20 days in suitable medium. C: The multiple shoots induced from the buds in after 15 days, the optimal formula of culture medium of *S. sebiferum* bud proliferation is: MS + 6-BA1.0mg/L+KT 0.5mg/L + NAA0.5mg/L. D: Root culture of single seedling. E: The average root length was 2.5cm in the medium 1/2MS+0.5mg/L IBA F: Transplanted seedlings survived after 25 days.

TABLE 2
EFFECT OF DIFFERENT MEDIUM ON THE EFFECTIVE BUD INDUCTION RATE OF STEM SEGMENT WITH AXILLARY BUD

Treatment number	medium	Hormone mg/L		Induction rate%	Activated days/d
		6-BA	NAA		
1	MS	0.5	0	44.4	4-6
2	MS	1.0	0.05	40.7	4-6
3	MS	2.0	0.10	33.3	4-6
4	WPM	0.5	0.05	62.9	3-5
5	WPM	1.0	0.10	45.7	3-5
6	WPM	2.0	0	42.9	4-6
7	B5	0.5	0.10	35.5	4-7
8	B5	1.0	0	32.1	5-7
9	B5	2.0	0.05	28.6	5-7

Analysis of variance showed that among the basic media, $P < 0.0001$, its F value was 16.89, far greater than $F_{0.01}(2, 20) = 5.85$; among the Ba mass concentration, $P < 0.0027$, its F value was 8.08, far greater than $F_{0.01}(2, 20) = 5.85$; among the NAA mass concentration, $P < 0.1877$, its F value was 1.82, less than $F_{0.05}(2, 20) = 3.49$. It can be seen that the effect of NAA concentration on axillary bud induction rate is not significant. The effect of basic medium and 6-BA concentration on axillary bud induction rate is extremely significant. Among the three basic media, WPM medium has the highest effective bud induction rate, which reflects that WPM medium is conducive to the induction and growth of axillary buds of woody plants.

There is significant difference in axillary bud induction rate among MS, WPM and B5 medium levels (Table 3). The average induction rate was 51.50% at WPM medium. When the mass concentration (MC) of 6-BA was 0.5mg/L, the average induction rate was 47.60%, which was significantly higher than the other two levels. and there was no significant difference between the concentrations of 1.0 and 2.0mg/L. So, 0.5mg/L 6-BA has the best induction effect on the stem segment of *S. sebiferum*. There was no significant difference in NAA concentrations. However, when the mass concentration of NAA is 0.05mg/L, the average induction rate is 44.07%, which is better than the other two levels. Therefore, the most favorable medium combination for effective bud induction is WPM + 6-BA0.5mg/L + NAA0.05mg/L.

TABLE 3
MULTIPLE COMPARISON OF EFFECTIVE BUD INDUCTION OF STEM SEGMENT WITH AXILLARY BUD

Medium	Average rate of induction	6-BA		NAA	
		Mc mg/L	Average %	Mc mg/L	Average %
MS	39.47 ^b	0.5	47.60a	0	39.80a
WPM	51.50 ^a	1.0	39.50b	0.05	44.07a
B5	32.07 ^c	2.0	34.93b	0.10	38.17a

3.3 The effect of different hormone combinations on the axillary bud proliferation of *S. sebiferum*.

In the subsequent generation proliferation test, the cut shoots were cultured for proliferation (Fig.1-C), the growth of the shoots was observed and proliferation was counted after 25 days, as shown in Table 4. the proliferation rate of medium 3 and 5 is good, 78.5% and 81.2% respectively, and their multiplication times 2.7 and 3.2 are also higher than other medium,

The worst medium 9, only 51.3% of buds proliferated, and the proliferation coefficient was only 2.1. The results of variance analysis showed that 6-BA、KT (Kinten) and NAA concentration had a significant effect on bud proliferation rate, F value was 11.83, 9.16 and 38.32 respectively, P value was less than 0.0004, 0.0015 and 0.0001 respectively. It can be seen from the F values of 6-BA, KT and NAA that NAA is the main factor affecting bud proliferation, followed by 6-BA.

TABLE 4
RELATIONSHIP BETWEEN AXILLARY BUD PROLIFERATION RATE, PROLIFERATION COEFFICIENT AND HORMONE RATIO IN SUBCULTURE

Treatment	Hormone ratio mg/ L			Proliferation rate %	Proliferation coefficient%
	6-BA	KT	NAA		
1	0.5	0.1	0	52.3	2.1
2	0.5	0.5	0.1	51.4	2.0
3	0.5	1.0	0.5	78.5	2.7
4	1.0	0.1	0.1	54.9	2.2
5	1.0	0.5	0.5	81.2	3.2
6	1.0	1.0	0	72.4	2.5
7	2.0	0.1	0.5	63.5	2.4
8	2.0	0.5	0	58.7	2.2
9	2.0	1.0	0.1	51.3	2.1

Table 5 showed the multiple comparison results of the effects of 6-BA, KT and NAA concentration on the proliferation rate of bud proliferation culture. When the concentration of 6-BA was 1.0mg/L, the average proliferation rate was 69.50%, which was significantly higher than the other two levels. When the concentration of KT is 0.5 and 1.0mg/L, it is also significantly higher than 0.1mg/L, but not between 0.5 and 1.0mg/L. Considering the high price of KT, 0.5mg/L is the best. The average proliferation rate was 74.33% when the concentration of NAA was 0.5mg/L.

TABLE 5
MULTIPLE COMPARISON OF THE EFFECTS OF 6-BA, KT AND NAA ON THE PROLIFERATION RATE OF BUD PROLIFERATION CULTURE

6-BA		KT		NAA	
MC mg/L	Average%	MC mg/L	Average%	MC mg/L	Average%
0.5	60.73b	0.1	56.83b	0	61.13b
1.0	69.50a	0.5	63.77a	0.1	52.53c
2.0	57.77b	1.0	67.40a	0.5	74.33a

The analysis of variance of multiplication coefficient of shoot proliferation culture showed that there was no significant difference between the three factors of 6-BA, KT and NAA concentration on the multiplication coefficient of *S. sebiferum* shoot (table omitted), which indicated that the three levels of 6-BA, KT and naa3 had little effect on the multiplication coefficient. Therefore, considering the proliferation rate, the most favorable medium combination was MS + 6-BA 1.0mg/L+KT 0.5mg/L + NAA 0.5mg/L.

3.4 The effect of IBA concentration on the rooting of *S. sebiferum*.

This experiment used 1/2MS medium with a large number of elements halved, selected IBA as exogenous hormone, inoculated about 1.5 cm stems in rooting medium, cultured under light. Generally, about 10 days, the base of the stem was swollen, and white root buds can be observed. After 30 days, the rooting rate, rooting multiple and rooting length were counted (Fig.1-D). As shown in table 6, it was found that the mass concentration of IBA had a great influence on the rooting. The rooting rate and rooting number of the 3 treatments were significantly different. Among them, the mass concentration of IBA was 0.5mg/L, the highest rooting rate was 93.3%; the average rooting multiple was 3.2, and a few bases produced callus. When the IBA mass concentration was 1.0 mg/L, the base was prone to produce a large number of callus, only straight roots, and thick, and some pseudo roots (invalid roots). Treatment 3, the rooting rate was relatively low. It can be seen that in the tissue rooting process of *S. sebiferum*, the IBA mass concentration is better with 0.5mg/L (Fig.1-E).

TABLE 6
EFFECT OF IBA ON ROOTING OF *S. sebiferum*

NO.	MC mg/L	Rooting rate %	Rooting multiple	root length
1	0.1	84.4Bb	2.7b	1.7b
2	0.5	93.3Aa	3.2a	2.5a
3	1.0	71.8Bc	1.8c	2.0b

3.5 Transplanting of rooting seedlings for sterile seedlings with strong rooting growth.

The sealing film on the bottle will be unraveled first, and the sealing film will not be removed. After 1 day, the sealing film will be completely removed and opened for 1-2 d; then, the seedlings were taken out and the medium on the roots was washed. The roots were further immersed in 0.2% carbendazim solution for 3 minutes and then transplanted into a seedling substrate of m (river sand): m (nutrient soil) = 1:1. In the environment where the temperature was about 25 °C and the humidity was maintained at 90% or more, the survival rate was 83.3% at 30 days (Fig.1-F).

IV. CONCLUSIONS AND DISCUSSION

The modernization of plant biotechnology began at the beginning of the 20th century, Plant tissue cell culture and regeneration, and cloning of new plants. After that, through molecular breeding to modify their genetic characteristics, including marker-assisted selection, genetic transformation and gene editing, there are likely to be more new programs in the future (Altman 2019). However, as a basic technology, tissue culture has made a great contribution. Among them, micropropagation technology has the advantages of rapid propagation, maintaining in vitro activity and protecting germplasm resources (Amirchakhmaghi et al. 2019). Just to solve the problem that some woody plants are difficult to be sowed or cut propagation and the vitality is easy to degenerate. Difficulty in sowing or cutting propagation.

During the cultivation of *S. sebiferum* stem as explant, the pollution is more serious. After 30 s of disinfection with 70% alcohol and 10 minutes of sterilization with 0.1% mercuric chloride, the pollution rate is still high, which may be related to endophytic bacteria. In many woody plant species, especially in the wild, bacterial contamination is particularly difficult to treat and detect, because bacterial infection is large and often cannot be accurately identified and disposed in plant tissues (Viss et al.1991; Buckley et al. 1995). Antibiotics can be added to the culture medium to prevent the pollution of stem culture, which may needs further study. Ascorbic Acid (VC), Polyvinyl Pyrrolidone (PVP) or Sodium thiosulfate pentahydrate (SH) can be added to the medium optionally to control browning.

The success of tree tissue culture system largely depends on the selection of medium (Liu et al. 2017). WPM medium is conducive to the induction and growth of axillary buds of woody plants. It has been reported that the possibility of variation in vitro is much lower through axillary bud and adventitious bud as explants (Sharma et al. 2007; Shenoy and Vasil 1992). In this experiment, stem segment with buds of *Sapium sebiferum* was used as explant, and buds were induced in medium of WPM + 6-BA0.5mg/L + NAA 0.05mg/L. The induction rate was 62.9% after 30 days. In the 3 levels of 6-BA concentration, when the concentration was 0.5mg/L, the average induction rate was 47.60%, significantly higher than the other two levels, it is also confirmed that 6-BA, at high concentration can stimulate shoot multiplication and inhibit their elongation (Lee and Wetzstein 1990; Jaime and Teixeira 2003). The variety and combination of growth regulators are more comprehensive, which is beneficial to screening of culture conditions conductively. Multiple analysis also be more scientific selection.

In the process of proliferation, the medium of MS + 6-BA1.0 mg/L + KT 0.5mg/L + NAA0.5mg/L had the best proliferation effect, and the multiplication multiple was up to 3.2. IBA is the most commonly used for rooting induction (Keresa et al. 2012; Xu et al. 2008). Single bud was inoculated on 1/2MS + IBA 0.5mg/L medium for rooting induction, with the best effect and the rooting rate of 93.3%. These results showed that the concentration of hormones used in the medium significantly affected the regeneration mode and differentiation type of the culture. In our study, axillary bud stem segment was used as explants, there is recent scientific study (Hou 2020) on the establishment of in vitro plant regeneration systems directly derived from leaves and petioles of *S. sebiferum*, and adventitious bud induction rate is very high. This study has completed the study of aseptic culture of tissues, and the next step is the study of cell suspension culture and secondary metabolites, which is devoted to the identification of chemical components and pharmacological analysis of cell products, and in the hope of contributing to human health and forest bioenergy.

LIST OF ABBREVIATIONS

Abbreviation	Full name
6-BA	6-benzyladenine
NAA	Naphthalene acetic acid
KT	Kinten
HgCl ₂	Mercuric Chloride

ACKNOWLEDGEMENTS

This study was supported by the National Natural Science Foundation of China (GrantNo.31971584).

The authors declare no competing interests.

REFERENCES

- [1] Chinese Academy of Science. 1980. *Iconographia Cormophytorum Sinicorum Tomus II* (ed. Institute of botany, Chinese Academy of Science). Beijing : Beijing Science Press.
- [2] Li, H., Yang, Z.B. Hu, J. 2011. Summary of exploitation on *sapium sebiferum* (L.) Roxb. Seeds. Hubei forestry technology 6: 42-45.
- [3] Fu, R., Zhang, Y.T., Peng, T., Guo, Y.R., Chen, F. 2015. Phenolic composition and effects on allergic contact dermatitis of phenolic extracts *Sapium sebiferum* (L.) Roxb. leaves. Journal of ethnopharmacology 162: 176-180.
- [4] Li, X.L., Liu, J.L. 1994. The Interrelation study between the tallow yield, quality and geographical dtribution of the Chinese vegetable tallow tree. Journal of East China Normal University(Ntural Science) 102-108 .
- [5] Jin, D.J., Huang, H.K., Tang, R.Q., Tong, Q.Y., Shi, D.Y., Hou, Z.H.. 1997. THE INVESTIGATION ON THE VARIETY RESOURCES OF SAPIUM SEBIFERUM IN CHINA. Guihaia 17: 58-75.
- [6] Shi, H., Guo, H.2001. Development and prospect of manufacturing CBE with Chinese tall oil. China Oils and Fats 6: 91-94.
- [7] Tan, Z.Q. 2013. Studies of the preparation of low-calorie Chinese Tallow cocoa butter equivalent by Lipase-catalyzed interesterification. [Master's thesis], South China university of technology.
- [8] Li, X.Q., Xu, Y.M. 2006. Discussion on development of major oleiferous tree species in China and their Energy forest construction. Biomass Chemical Engineering 229-234.
- [9] Peng, C. 2013. Biodiesel production from Chinese tallow kernel oil and its combustion, emission properties. [Master's thesis], Huazhong university of science and technology.
- [10] Wu, K.J., Huang, Y., Xiao, X.X., He, W.G., Zhang, B. 2011. Research on biodiesel production from Tallow kernel oil by acid-alkali catalysis process. Jour of Fujian Forestry Sci and Tech 38: 62-64+68.
- [11] Helwani, Z., Othman, M.R., Aziz, N., Fernando,W.J.N., Kim, J. 2009. Technologies for production of biodiesel focusing on green catalytic techniques: A review. Fuel Processing Technology 90:1502-1514.
- [12] Chen, W.W. 2006. Research of Biodiesel Production from Chinese Tallow Kernel Oil. [Master's thesis], Nanchang University.
- [13] Campanelli, P., Banchemo, M., Manna, L. 2010. Synthesis of biodiesel from edible, non-edible and waste cooking oils via supercritical methyl acetate transesterification. Fuel 89: 3675-3682.
- [14] Hayyan, M., Mjalli, F.S. 2010. Hashim MA, AlNashef IM. A novel technique for separating glycerine from palm oil — based biodiesel using ionic liquids. Fuel Processing Technology 91: 116-120.
- [15] Venketeswaran, S., Gandhi, V., Romano, E.J., Nagmani, R. 1983. Mass Propagation of Selected Trees for Biomass by Tissue Culture. Biomass Utilization. NATO Advanced Science Institutes Series (Series A: Life Sciences). 67: 191-204.
- [16] Bajaj, Y.P.S. 1986. Biotechnology of Tree Improvement for Rapid Propagation and Biomass Energy Production. In: Bajaj Y.P.S. (eds) Trees I. Biotechnology in Agriculture and Forestry 1. Springer, Berlin, Heidelberg.
- [17] Bommineni, V.R., Mathews, H., Samuel, S., Kramer, M., Wagner, D. 2001. A new method for rapid in vitro propagation of apple and pear. Hort Science 36: 1102-1106.
- [18] Venketeswaran, S., Gandhi, V. 1982. Mass propagation and genetic improvement of forest trees for biomass production by tissue culture. Biomass 2: 5-15.
- [19] Siril, E.A., Dhar, U. 1997. Micropropagation of mature Chinese tallow tree (*Sapium sebiferum* Roxb.). Plant Cell Reports 16: 637-640.
- [20] Altman, A. 2019. Plant tissue culture and biotechnology: perspectives in the history and prospects of the International Association of Plant Biotechnology (IAPB). In Vitro Cellular & Developmental Biology– Plant 55: 590-594.
- [21] Amirchakhmaghi, N., Hosseinpour, B. Yousefzadeh, H. 2019. Development of a micropropagation protocol for *Malus orientalis* using axillary buds. In Vitro Cellular & Developmental Biology – Plant 55: 625-634.
- [22] Viss, P.R., Brooks, E.M., Driver, J.A.1991. A simplified method for the control of bacterial contamination in woody plant tissue culture. In vitro Cell Dev Biol Plant 27: 42.
- [23] Buckley, P.M., Dewild, T.N., Reed, B.M.1995. Characterization and identification of bacteria isolated from micropropa gated mint plant. In Vitro Cell Dev Biol Plant 31: 58-64.

- [24] Liu, C.P., Liu, H.Y., Yang, B.W., Yang, L., Zhang, P., Shen, H.L. 2017. Shoot multiplication of *Syringa reticulata* var. *mandshurica* from in vitro cultured seedlings. *Journal of Forestry Research* 28: 41-46.
- [25] Sharma, S., Bryan, G., Winfield, M., Millam, S. 2007. Stability of potato (*Solanum tuberosum* L.) plants regenerated via somatic embryos, axillary bud proliferated shoots, microtubers and true potato seeds: a comparative phenotypic, cytogenetic and molecular assessment. *Planta* 226: 1449-1458.
- [26] Shenoy, V.B., Vasil, I.K. 1992. Biochemical and molecular analysis of plants derived from embryogenic tissue cultures of napier (*Pennisetum purpureum* K. Schum). *Theor Appl Genet.* 83: 947-955.
- [27] Lee, N., Wetzstein, H.Y. 1990. In vitro propagation of muscadine grape by axillary shoot proliferation. *J Am Soc Hortic Sci* 115: 324-329.
- [28] Jaime, A., Teixeira da, S. 2003. Chrysanthemum: advances in tissue culture, cryopreservation, postharvest technology, genetics and transgenic biotechnology. *Biotechnology Advances* 21: 715-766.
- [29] Keres, S., Bosnjak, A.M., Baric, M., Jercic, I.H. 2012. Sarcevic H, Bisko A. Efficient axillary shoot proliferation and in vitro rooting of apple cv. 'Topaz'. *Not Bot Horti Agrobo* 40: 113-118.
- [30] Xu, J., Wang, Y.Z., Zhang, Y.X., Chai, T.Y. 2008. Rapid in vitro multiplication and in vitro rooting of *Malus zumi* (Matsumura) Rehd. *Acta Physiol Plant* 30:129-132.
- [31] Hou, J.Y. 2020. Efficient plant regeneration from in vitro leaves and petioles via shoot organogenesis in *Sapium sebiferum* Roxb. *Plant Cell, Tissue and Organ Culture (PCTOC)*. 142: 143-156. <https://doi.org/10.1007/s11240-020-01848-9>.