Bioscience Method 2023, Vol.14, No.3, 1-9
http://bioscipublisher.com/index.php/bm

Research Article Open Access

Establishment of Tissue Culture and Rapid Propagation System on *Mandevilla sanderi*

Zhang Yueyuan, Hu Mengxuan, Di Bao, Chen Duanfen
College of horticulture, Hebei Agricultural University, Baoding, 071001, China
Corresponding author email: chenduanfen@163.com

Bioscience Method, 2023, Vol.14, No.3 doi: 10.5376/bm.2023.14.0003
Received: 11 Apr., 2022
Accepted: 05 Apr., 2023
Published: 06 May, 2023

Preferred citation for this article:
Zhang Y.Y., Hu M.X., Di B., and Chen D.F., 2023, Establishment of tissue culture and rapid propagation system on *Mandevilla sanderi*, Bioscience Method, 14(3): 1-9 (doi: 10.5376/bm.2023.14.0003)

Abstract Aimed at the problems of low reproductive coefficient and long seeding period for *Mandevilla sanderi* 'Sunparasol', the rapid propagation system was studied in this article. Research results showed as follows: The disinfectant and disinfection time for young stem segments of *M.sanderi* is 0.1% $HgCl_2$ for 3 min; When the culture medium is MS+6-BA 4 mg/L+NAA 0.1 mg/L, the germination rate is as high as 93.33%; In the subculture stage, the number of multiplication and growth is strong in the medium of MS+6-BA 2 mg/L+NAA 0.1 mg/L, and the multiplication coefficient is 4.31; Sucrose concentration of 40 g/L can promote the growth of fine tissue culture seedlings; the optimum growth condition of test tube seedlings is the best when 1~2 mg/L of CCC is added; The suitable rooting medium is 1/2MS+NAA 0.1 mg/L, the rooting rate is 83.33%, and the average rooting number is 4.3. The above-mentioned establishment of tissue culture rapid propagation system has important guiding significance for the large-scale production of *M.sanderi*.

Keywords *Mandevilla sanderi*; Tissue culture; Rapid propagation system

Mandevilla Sanderi, also known as fragrant vine, is a perennial evergreen vine belonging to the genus Mandevilla in the Apocynaceae. *Mandevilla Sanderi* with large and colorful flowers and elegant branches. It can not only be used as indoor potted plants for home decoration on balconies, windowsills, corridors and steps, but also for the beautification of hedge walls, trellises, rooftops and small courtyards, with high ornamental value (Wu, 2016). In addition, the natural essential oil extracted from the flower can refresh the mind and regulate mood; Mandevilla plants contain bioactive substances that can be used to treat inflammation and other diseases and have great potential in treating cardiovascular diseases (Biondo et al., 2007; Waléria et al., 2010; Souza et al., 2011; Ferreira et al., 2017). Therefore, the *Mandevilla sanderi*, known as the "Queen of Tropical Vines", is an ornamental and medicinal plant with great market potential (Xue, 2017).

The cuttage method is commonly used for propagation of *Mandevilla sanderi*, and it takes about 3 months to emerge under normal water and fertilizer conditions (Lai et al., 2011). This propagation method not only has a low propagation coefficient and a long production cycle, but also fails to meet the market demand for seedlings due to seasonal restrictions, pest infestation and other reasons (Wu et al., 2007). Tissue culture can obtain a large number of high quality pest-free seedlings in a short time, but there are few reports on tissue culture of *Mandevilla sanderi*. Therefore, it is of great significance to establish a perfect tissue culture and rapid propagation system for the large-scale production of *Mandevilla saderi*. Chen (2014) found that the top bud of twigs of *Mandevilla sanderi* could be used as explants for tissue culture to obtain sterile seedlings, but the top bud explants were too young and difficult to be sterilized, and the rate of seedling formation was low. Some studies have shown that rapid propagation of the above materials can be achieved by using the lateral bud stem segments of *Crossostephium chinense* (Chen et al., 2012) and *Hydrangea paniculata* (Wang et al., 2020) for tissue culture, but there are few reports on the induction differentiation and rooting culture of the lateral bud stem segments of *Mandevilla sanderi* as explants. In this study, the previous research methods were used for reference and improvement, aiming at establishing a complete culture system for the plant group of *Mandevilla sanderi*, and providing technical support for the large-scale production of *Mandevilla sanderi*.

1 Results and Analysis

1.1 Effects of different concentrations of $HgCl_2$ and disinfection time on stem segments

The stem segment was tested with different concentrations of $HgCl_2$ and disinfection time, and the results showed (Table 1) that 0.1% $HgCl_2$ had the best disinfection effect on young explants, followed by 0.15% $HgCl_2$. The mortality of stem segment increased with time. In the same treatment time, the survival rate of 0.1% $HgCl_2$ is higher than that of the other two $HgCl_2$ concentrations. When the treatment time of different concentrations was 7 min, the mortality rate was more than 30%. Therefore, 0.1% $HgCl_2$ disinfection for 3 min was the appropriate disinfection method, the survival rate of explants was 73.33%, and the contamination rate was only 7.78%.

Table 1 Comparison of sterilization effects of different sterilizers and their processing time on young stems

Disinfectors	Sterilization time (min)	Contaminated rate (%)	Death rate (%)	Survival rate (%)
$HgCl_2$ (0.05%)	3	57.78 ± 2.32^{a}	27.78 ± 1.67^{cde}	24.44 ± 0.63^{cd}
	5	37.78 ± 2.23^{ab}	31.11 ± 2.15^{bc}	36.67 ± 2.59^{cd}
	7	50.00 ± 3.56^{bc}	35.56 ± 3.12^{bc}	21.11 ± 4.14^{cd}
$HgCl_2$ (0.1%)	3	7.78 ± 1.41^{e}	6.67 ± 2.10^{e}	73.33 ± 3.70^{a}
	5	25.56 ± 1.53^{de}	15.56 ± 3.08^{de}	51.11 ± 4.01^{ab}
	7	17.78 ± 3.19^{cde}	31.11 ± 2.67^{a}	47.78 ± 4.32^{cd}
$HgCl_2$ (0.15%)	3	22.22 ± 2.89^{bc}	28.89 ± 1.08^{cd}	37.78 ± 0.76^{bc}
	5	27.78 ± 3.42^{bc}	38.89 ± 0.99^{abc}	40.00 ± 1.58^{cd}
	7	41.11 ± 4.11^{bcd}	44.44 ± 1.95^{ab}	28.87 ± 0.86^{d}

Note: The variance analysis of different lowercase alphabets in the table was significantly different ($P<0.05$)

1.2 Start-up culture

Stem segments with axillary buds were selected as explants in start-up culture. Different hormone ratios and culture results showed (Table 2) that when 6-BA concentration was 4 mg/L and NAA concentration was 0.1 mg/L, the sprouting rate of explants was the highest, and the axillary buds in stem segments began to sprout about 7 days after inoculation (Figure 1A; Figure 1B), axillary bud elongation was about 1 cm at about 15 d (Figure 1C). As the axillary buds grew, light green callus arose in the lower part of the stem segment (Figure 1D). In blank MS medium, it was about 15 days before the explants showed signs of germination, and the growth was weak after germination. After growing for 30 days (Figure 1E; Figure 1F), obvious internodes can be seen, and subculture can be carried out.

Table 2 Effects of different hormones on the germination and differentiation of explants

6-BA (mg/L)	NAA (mg/L)	No. of explant	Sprouting time (d)	No. of germination	Sprouting rate (%)
0	0	30	15	3.67	36.67 ± 3.69^{de}
2	0.1	30	10	7.67	76.67 ± 1.84^{b}
	0.3	30	10	4.67	46.67 ± 3.52^{cd}
	0.5	30	11	4.33	43.33 ± 2.21^{cd}
4	0.1	30	7	9.33	93.33 ± 3.82^{a}
	0.3	30	11	2.67	26.67 ± 0.58^{e}
	0.5	30	12	5.00	50.00 ± 4.14^{c}
6	0.1	30	9	4.33	43.33 ± 2.73^{cd}
	0.3	30	9	3.67	36.67 ± 1.80^{de}
	0.5	30	12	1.33	13.33 ± 0.79^{f}

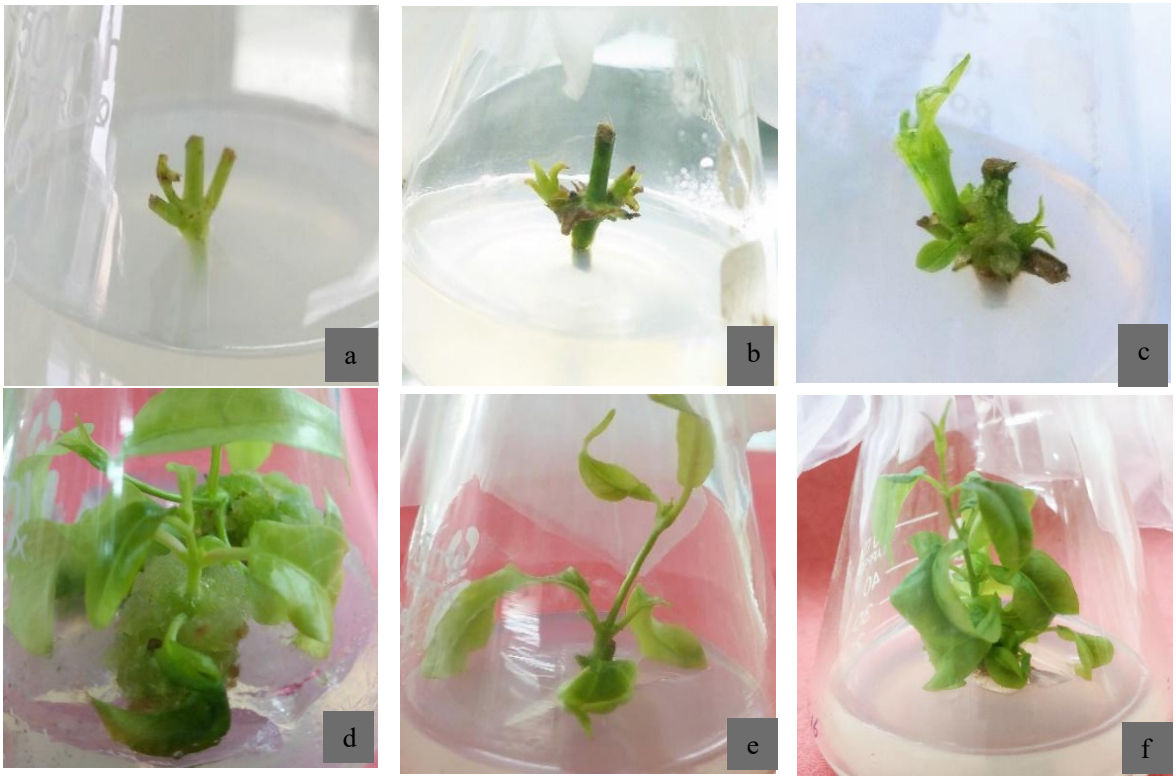

Figure 1 Germination and differentiation of explants

Note: a: Young explants; b: Young explant germination; c: Stem segment 15 d growth; d: Callus formation at the bottom of test-tube seedlings; e~f: Axillary bud elongation

1.3 Subculture

The results of subculture showed (Table 3) that, with the gradual increase of 6-BA concentration, callus at the bottom of seedlings would affect the proliferation and growth of the upper seedlings at the growth stage (Figure 2A). When no hormone was added in MS medium, the growth of tissue cultured seedlings was the weakest, and they would drop leaves and wither due to lack of nutrition in the growth process (Figure 2B). After comprehensive consideration, the optimal supplemental level of 6-BA and NAA for the proliferation of *Mandevilla sanderi* was 2 mg/L and 0.1 mg/L, indicating the optimal growth state (Figure 2C).

Table 3 Effects of different hormone ratios on the proliferation of tissue culture

6-BA (mg/L)	NAA(mg/L)	No. of explants	Multiplication coefficient	Growth of reproduced plant
0	0	30	1.30 ± 0.03^{i}	+
1	0.01	30	2.33 ± 0.52^{h}	+
	0.10	30	2.80 ± 1.00^{f}	++
2	0.01	30	3.28 ± 0.74^{d}	++
	0.10	30	4.31 ± 0.73^{a}	+++
3	0.01	30	3.12 ± 1.02^{e}	++
	0.10	30	3.43 ± 0.71^{c}	++
4	0.01	30	2.62 ± 0.20^{g}	+
	0.10	30	3.70 ± 0.17^{b}	++

Note: +++: Means for strong growth, leaves flat; ++: Growth general, leaf curl; +: Plant short, leaf curled; The variance analysis of different lowercase alphabets in the table was significantly different ($P<0.05$)

Figure 2 Proliferation of tissue culture
Note: a: 6-BA concentration is too high growth; b: No hormone culture added; c: Well-grown seedling

1.4 Cultivation of strong seedlings

1.4.1 Effect of different sucrose concentration on rejuvenation of tissue culture seedlings

The growth of tissue culture seedlings of *Mandevilla sanderi* with poor growth condition was significantly affected by adjusting sucrose concentration in medium supplemented with 6-BA (2 mg/L) and NAA (0.1 mg/L). In terms of appearance and morphology (Table 4), the tissue cultured seedlings under sucrose treatment of 20~40 g/L all grew normally (Figure 3A), showing obvious internodes and robust growth. When sucrose concentration was low (10~20 g/L), some tissue cultured seedlings showed slight vitrification (Figure 3B). When sucrose concentration is too high (50 g/L), leaves and internodes of the plant turn red (Figure 3C). Combined with the average seedling height of tissue culture plantlets (Table 4), it indicated that the delicate tissue culture plantlets could grow robustly by appropriately increasing sucrose concentration in the medium.

Table 4 The effect of sucrose on the rejuvenation of tissue culture

Treatment	Concentration of sucrose	Average seedling height (cm)	Growing state
1	10	0.91±0.04^{c}	Narrow and small leaves with unobvious internodes
2	20	1.97±0.50^{b}	Flat leaves and obvious internodes
3	40	2.19±0.33^{a}	Luxuriant growth with flat leaves
4	50	1.80±0.16^{b}	Leaves and internodes are red

Note: The variance analysis of different lowercase alphabets in the table was significantly different (P<0.05)

1.4.2 Effects of different concentrations of chlorocholine chloride (CCC) on strong seedlings in tissue culture

After adding CCC for culture, the tissue cultured seedlings had flat leaves, obvious internodes, thickened stems and vigorous growth (Figure 3D). When the supplemental level of CCC was greater than 5 mg/L, there were more callus at the bottom, which affected the growth of the tissue cultured seedlings at the top (Figure 3E). When the supplemental level was 1~2 mg/L, the upper part of the seedlings grew strong, and some seedlings had 2~3 roots (Figure 3F). Therefore, it can be added appropriately to strengthen seedlings, which is beneficial to the normal growth of plants and their subsequent rooting and transplanting.

1.5 Rooting culture

1.5.1 Effects of different basic media on rooting of tissue cultured seedlings

After 30 days of culture, the rooting rate and growth of tissue cultured seedlings were calculated. The results showed that (Table 5; Figure 4), when the mass element was 1/2MS, the rooting rate was 86.67%, and the average number of rooting was 6.3, which was significantly higher than that of other treatments, indicating that the basic medium suitable for rooting culture of *Mandevilla sanderi* was 1/2MS. The time of root emergence in the three media was about 7 days.

1.5.2 Hormone screening suitable for rooting

The results of rooting hormone screening test showed (Table 6) that the addition of 0.1 mg/L NAA had the highest rooting rate, the highest average number of roots and the longest average root length. Using 1/2MS as the basic medium, adding 0.1~1 mg/L NAA could significantly improve the rooting coarseness of tissue cultured seedlings (Figure 5). When the concentration of NAA was 0.1 mg/L, the rooting quality of tissue culture seedlings was the

best. When NAA was 0.1 mg/L, only root primordium appeared in tissue culture seedlings at 35 days, and no obvious root formation was observed.

1.6 Seedling refining and transplanting

Tissue culture seedling is always in the best growth environment, once out of the bottle transplanting, its growth environment has a great change, its growth will be affected. Correct seedling refining and transplanting is the key to directly affect the survival rate of transplanting seedlings (Xiong and Wu, 2002). Therefore, seedling refining is an important step to improve the adaptability of seedlings to the changeable environment. When the rooting bottle seedling height reached 4~5 cm, put the bottle seedling in the greenhouse seedling bed, maintain the light intensity of about 5000 Lx, the ambient temperature of 23℃~25 ℃, transition exercise for about 2 weeks, in order to enhance the seedling adaptability to the environment. One week before the bottle is put out, gradually open the cap for bottle opening exercise, that is, first loosen the cap for 1-2 days, then partially open the cap for 1~2 days, and finally remove the cap completely, so that the bottle seedlings gradually adapt to external light and humidity. After coming out of the bottle, the root culture medium was washed with clear water, and then the root was soaked with carbendazim 800 times for 10 min. After drying the water in the root, the seedlings were transplanted into the substrate with vermiculite: peat: perlite 3:1:1. The transplanted seedlings grew well (Figure 6), and the survival rate was 90%.

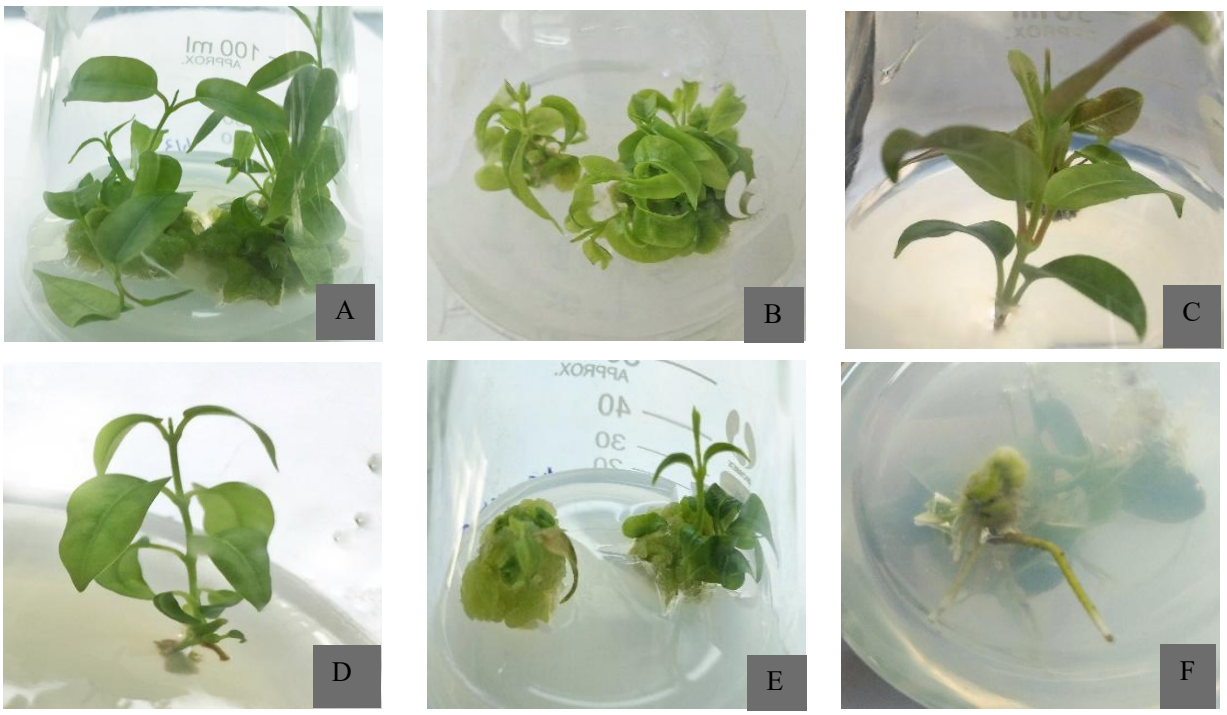

Figure 3 Strong seedling culture

Note: A: Effect of 30~40 g/L sucrose on the growth of plantlets; B: Effect of 20 g/L sucrose on the growth of plantlets; C: Effect of 50 g/L sucrose on the growth of plantlets; D: Treatment of robust plantlets with CCC; E: *In vitro* plantlets treated with high concentration of CCC; F: Roots appeared after 30 days of CCC treatment

Table 5 Effect of different types of basic medium on the rooting of the plantlets

Basic medium	Average rooting rate (%)	Average number of root	Average length of root (cm)	Growing situation
1/2MS	86.67±5.34[a]	6.3±0.53[a]	3.73±0.41[a]	Strong roots with some roots
1/3MS	53.33±5.81[b]	4.2±0.82[a]	3.31±0.83[a]	Stronger roots with more roots
1/4MS	40.00±3.43[b]	1.9±0.03[b]	1.40±0.07[b]	Weak roots with few roots

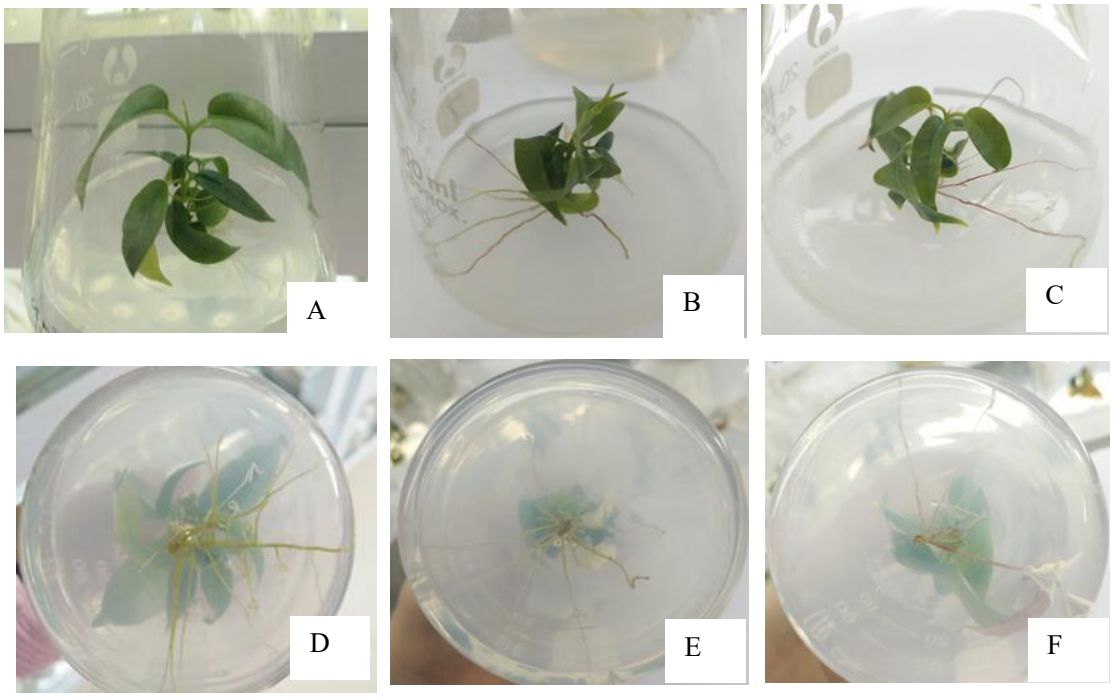

Figure 4 Effects of different basic media on rooting

Note: A,D: The rooting of 1/2 MS medium; B,E: The rooting of 1/3 MS medium; C,F: The rooting of 1/4 MS medium

Table 6 Effect of different concentration of NAA on the rooting

NAA (mg/L)	Average rooting rate (%)	Average number of root	Average length of root (cm)	Growing situation
0.1	83.33±6.32[a]	4.33±0.68[a]	1.50±0.22[a]	Thick roots with fast rooting
0.5	73.33±4.58[a]	2.33±0.17[b]	1.23±0.30[a]	Thicker roots with fast rooting
1	6.67±0.54[b]	0.33±0.04[c]	0.17±0.01[b]	Short roots with slow rooting

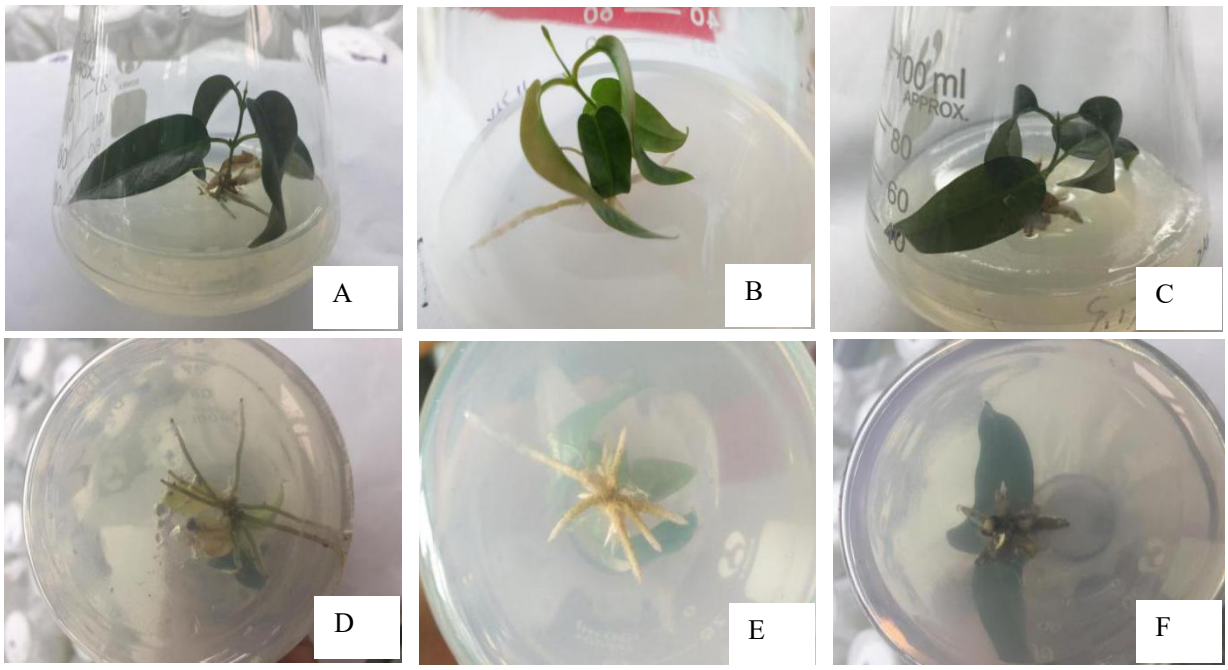

Figure 5 Effect of NAA concentration on rooting of tissue cultured seedlings

Note: A,D: The rooting of 0.1mg/L NAA added; B,E: The rooting of 0.5mg/L NAA added; C,F: The rooting of 1.0mg/L NAA added

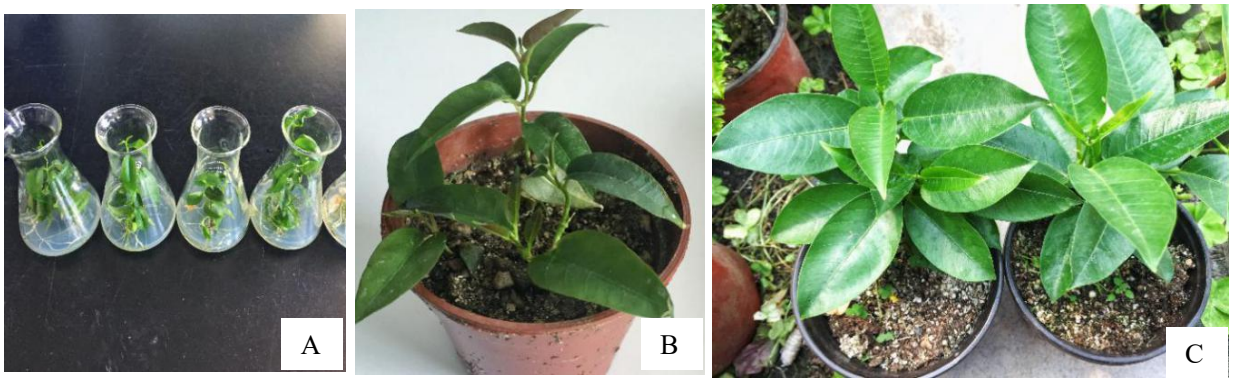

Figure 6 Seedling refining and transplanting
Note: A: Opening bottle film for hardening; B: Planting 35 d growth; C: Planting 90 d growth

2 Discussion

Theoretically speaking, all plant cells have totipotency. Any organ, tissue, or single cell can be used as an explant. But in fact, different varieties of plants and organs have different abilities of differentiation, and the degree of difficulty of tissue culture will also be different. In this study, stem segments with axillary buds were selected as explants, because explants have sufficient material, which is conducive to large-scale operation. The causes of contamination of plant materials are complex, including bacteria carrying in explants and bacteria contamination caused by improper operation in tissue culture process (Li et al., 1999). The contamination rate of explants with bacteria can be reduced to the maximum from different aspects, such as the season, time and location of explants and the application of different disinfectants. The pollution generated during tissue culture operation can be completely overcome (Zhao et al., 2001, Journal of Henan Forestry Science and Technology, 21(2): 27-28) by improving the operation process, such as high-pressure sterilization tools and petri dishes, cleaning the inoculation room regularly, and using different methods for disinfection.

At the start-up stage of explants, the germination of axillary buds of plant materials was affected by different hormone ratios. The tissue culture medium was MS medium. Nine hormone ratios were designed in the initiation culture stage of *Mandevilla sanderi*, and the results showed that the addition of 6-BA at a high concentration affected the initiation of axillary buds, while the addition of NAA at a high concentration resulted in excessive callus formation and reduced germination rate, which was consistent with the results of Li et al. (2004) in the study of rapid propagation of *Mandevilla sanderi*. Multiplication coefficient is an important index to measure in subculture. The proliferation factor is influenced by many factors, such as culture medium and hormones. Wu et al. (2007) added a relatively high concentration of 6-BA in the rapid propagation culture of *Mandevilla sanderi* at the initial stage of culture, but with the increase of culture algebra, some seedlings became thin and produced large callus. The concentrations of 6-BA and NAA were decreased after multiple subgenerations. Li et al. (2004) showed that KT had no effect on the proliferation of *Mandevilla sanderi* in the rapid propagation culture. When 6-BA was 4 mg/L, the proliferation effect was the best. 6-BA was more suitable for subculture of *Mandevilla sanderi*. Different auxin concentrations also affected the proliferation coefficient. When NAA concentration was higher, callus growth hindered the growth of tube seedlings. In this study, the suitable addition level of 6-BA was 2 mg/L, which was consistent with the previous studies (Li et al., 2004; Wu et al., 2007). This may be caused by large differences in growth environment and internal physiological and biochemical indexes of plants of the same genus, or differences in culture conditions.

Aiming at the phenomenon of elongated stems and no obvious internodes in culture. Sucrose is a very important organic nutrient and can play its role. Therefore, increase and decrease sucrose concentration in the culture process to determine its role in the delicate seedling culture cycle. The results showed that when the amount of sucrose was low, the tissue culture seedlings still grew small and weak between 10 g/L and 20 g/L. When sucrose was added at a high concentration of 50 g/L, the petiole and neck of seedlings were flushed, but had no effect on growth. When the amount of sucrose was between 30 g/L and 40 g/L, the seedling growth accelerated and

gradually grew normally, with obvious internodes. The main role of CCC is to make plants dwarf (Fu et al., 2008). The growth condition of delicate seedlings was improved by adding CCC in this experiment. Therefore, by increasing the amount of sucrose and adding 1~2 mg/L CCC, the normal growth of delicate seedlings can be achieved.

In the selection of the basic media for rooting of *Mandevilla sanderi*, it was found that 1/2MS, 1/3MS, 1/4MS were used to inoculate the tissue culture seedlings of primary culture on these three different basic media. The time of root emergence in the three bottles was about 7 days, and the root quality in 1/2MS was relatively good. In experimental studies of different concentrations of NAA hormone levels, it was found that the time of root emergence in 0.1 mg/L NAA bottle was 13 d, the time of root emergence in 0.5 mg/L NAA bottle was 17 d, and only the formation of root primordia occurred from 1.0 mg/L NAA bottle to 35 d. According to the experimental results, although the addition of 0.5 mg/L NAA could significantly improve the root quality, but delayed the root emergence time, and the study of the optimal ratio of hormone levels for the root will still be an important issue for future research.

3 Materials and Methods

3.1 Test materials

The tested material was "Sunparasol", which was purchased from Beijing Huamu Co., Ltd., and the stem segment of its young axillary bud was taken as the test material. The test place was conducted in the Inoculation Room and Tissue Culture Room of West Campus of Hebei Agricultural University.

3.2 Material treatment

Acquisition of primary sterile explants: Take vigorous growth of the *Mandevilla sanderi* stem with axillary bud period of 2~3 cm, with detergent solution soak for 10 min, rinse with tap water for 30 min, distilled water rinse three times. Then the stem segments were soaked in 75 % alcohol solution for 1 min on the ultra-clean worktable, rinsed with sterile water for 1 time, and then treated with different concentrations of mercuric chloride solution for a period of time, rinsed with sterile water for 4 times, cut into 1 cm stem segments, and inoculated on the initial medium according to the natural growth direction.

Initiation culture: 2-factor and 3-level orthogonal test of 6-BA and NAA combination was selected. The dosage of 6-BA was 2 mg/L, 4 mg/L and 6 mg/L. The supplemental levels of NAA were 0.1 mg/L, 0.3 mg/L and 0.5 mg/L. MS medium without any hormones was the blank control. Inoculated plant materials should be as consistent as possible. Each treatment was inoculated with 10 vials, repeated 3 times. After inoculation, the seedlings were exposed to fluorescent lamps of 1 000~2 000 lx at (25±2)℃ for 12 h and darkness for 12 h (The same below). Germination of the explants was recorded, and the number of germination buds was counted 30 days later.

Subculture: When the seedlings of the explants inoculated in the start-up medium grew to about 2 cm, the seedlings with relatively consistent growth status were cut and inoculated into the medium supplemented with 6-BA (0, 1, 2, 3, 4 mg/L) and NAA (0, 0.01, 0.1 mg/L), respectively. After inoculation, the effects of two factors on the proliferation of tissue culture seedlings were observed under appropriate environmental conditions. The germination number and growth status of seedlings were observed 30 days after inoculation.

Seedling culture: MS was used as the basic medium, 6-BA (2 mg/L), NAA (0.1 mg/L) was added. On this basis, the rejuvenation of delicate tissue culture seedlings was observed by increasing or decreasing sucrose concentration and adding CCC.

Rooting culture: select tissue culture seedlings with rejuvenation, seedling height of about 3 cm, dark green leaves, thick stems and relatively consistent growth status. They were inoculated in 1/2MS, 1/3MS and 1/4MS medium, respectively. Observe the the rooting days and its growth status. Based on the optimal medium, auxin NAA was added for rooting comparison with 0.05, 0.1, 0.2, 0.3 mg/L, respectively. After inoculation, the root quality, root length and number of tissue cultured seedlings were taken as indexes under appropriate environmental conditions.

Seedling refining and transplanting: the seedlings were removed from the bottle for transplanting after rooting culture for about 40 days. The AGAR residue of the roots was washed with water, and the roots were dipped in thiophanate methyl solution with appropriate concentration. The seedlings were planted in a mixed matrix, and proper ventilation was maintained during the growth process.

3.3 Test indicators

After the material was inoculated, the growth was observed and recorded, and the statistical indexes were as follows:

Contamination rate (%)=Number of contaminated explants/total number of inoculated explants×100%

Germination rate(%)=Number of germination explants/total number of uncontaminated and unbrowning lethal explants×100%

Multiplication coefficient =Total number of shoots at the end of multiplication cycle/total number of shoots at the beginning of multiplication cycle

Rooting rate (%)=(Number of rooting seedlings/total number of cultured seedlings)×100%

Average number of roots =Total number of roots of explants/number of seedlings

Average root length =Total rooting length/number of rooting seedlings

Author contributions

ZYY and HMX were the implementers of the experimental design and research of this study. ZYY and HMX completed data analysis and wrote the first draft of the paper. DB participated in experimental design and analysis of experimental results; CDF was the designer and principal of the project, directing experimental design, data analysis, paper writing and revision. All authors read and approved the final manuscript.

Acknowledgments

This study was supported by the Collaborative Innovation Project between Teachers and Students of Heibei Agricultural University (SSXT201705).

References

Waléria B.B., Valéria de S.A., Ronaldo B., C S.F., PC T.A., and S P.M.A., 2010, Genetic diversity among natural populations of *Mandevilla velutina*, Hortic Bras, 28(2): 209-213.
https://doi.org/10.1590/S0102-05362010000200012

Biondo R., Souza A.V., Bertoni B.W., Soares A., França S.C., and Pereira A.M.S., 2007, Micropropagation, seed propagation and germplasm bank of *Mandevilla velutina* (Mart.) Woodson, Sci. Agri., 64(3): 263-268.
https://doi.org/10.1590/S0103-90162007000300008

Chen X.J., Wu Y., Li X.K., Sun M., Zhang Q.X., 2012, Study on techniques of fast multiplication for the tissue culture of *Crossostephium chinense*, Zhongnan Linye Keji Daxue Xuebao (Journal of Central South University of Forestry & Technology), 32(7): 100-104.

Chen X.M., 2014, Analysis on the technique of transplanting flower plantlets *in vitro* by tissue culture, Xiandai Yuanyi (Modern horticulture), (10): 44.

Ferreira L.L.D.M., Gomes M.V., Paes B.M., do Carmo P.L., Konno T.U.P., Esteves F.A., Lopes N.P., Tomaz J.C.Tomaz I.C.R., Guimarães D.O., Muzitano M.F., and Raimundo J.M., 2017, The hydroalcoholic extract of leaves of Mandevilla moricandiana induces NO-mediated vascular relaxation, Planta Medica, 83(1-2): 63-69.
https://doi.org/10.1055/s-0042-108203

Fu H.L., He T.J., and Wu Q.Y., 2008, Research and application of plant growth regulators, Shengwu Jiagong Guocheng (Biological processing), (4): 7-12

Lai S.H., Lei J.L., Wu C.Q., and Xu Y.Y., 2011, Study on seedling propagation technology of new vertical greening varieties of overpass, Nanfang Nongye (Southern agriculture), 5(2): 37-40.

Li Q., Du W.P., Wang M.L., and Shi D.X., 1999, Study on pollution control technology in plant tissue culture, Sichuan Linye Keji (Sichuan Forestry Science and technology), 20(4): 22-26.

Li Y.H., Zeng D.X., and Xie L.J., 2004, Studies of the micropropagation of *Manderilla sanderi*, Linye Keji (Forestry science and technology), 29(5): 49-51

Souza, A.V., Bertoni B.W., Franca S.C., and Pereira A.M.S., 2011, *In vitro* conservation and rooting of "infalivel" (*Mandevilla velutina* K. Schum.), a medicinal plant of Cerrado, Rev. Bra. de Plantas Medicinais, 13(3): 319-327.
https://doi.org/10.1590/S1516-05722011000300011

Wu K.L., Zeng S.J., Chen Z.L., and Duan J., 2007, Tissue culture and rapid propagation of rattan, Zhiwu Shenglixue Tongxun (Plant Physiology Communication), 43(6): 1145-1146.

Wu Y., 2016, Double glandular rattan "Samba dance" series, Zhongguo Huahui Yuanyi (China Flower Gardening) (24): 32.

Xiong L., and Wu L.F., eds., 2002, Tissue culture and mass production of Ornamental flowers, Chemical Industry Press, Beijing, China, pp.5.