Research on Tissue Culture Rapid Propagation Technology of Taiwan Lily

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Abstract. This experiment selected Taiwan lily as experimental material to study the different ages of lily to produce different explants to induce differentiation, through subculture and the optimal conditions for tissue culture outdoor refining, and then the experimental staff established Systematic Taiwan lily flower tissue culture and rapid propagation technology. Studies have shown that the optimal medium for differentiation of Taiwan lily flower scales (winter sampling) is MS+0.2 ug/L GA3+0.1 mg/L NAA+1 mg/L 6-BA; optimal Taiwan lily leaves (winter sampling) The medium for differentiation was MS + 0.1 ug / L GA3 + 0.1 mg / L NAA + 1 mg / L KT. The best scale-induced differentiation medium for Taiwan lily (spring sampling) that was not in dormancy was MS+0.1 mg/L NAA+1.0 mg/L 6-BA, and the leaf differentiation medium was MS+0.1 mg/L NAA+ 1 mg/L IBA. MS+0.2 mg/L NAA+1.0 mg/L KT was the best callus differentiation bulb culture medium, MS+0.5 mg/L NAA+0.5 mg/L 6-BA was the best induction rooting medium.

1. Introduction

The lily growing in Taiwan is a native rare flower unique to Taiwan Province of China. The flowers of 1-10 flowers are arranged in a nearly umbrella shape. The flowers are white and trumpet-like, with aroma, and the stems are usually about 20-55cm high. It is relatively short in the species of the lily flower horn group. Therefore, in addition to being used for landscaping, it is also suitable for potted flower cutting and has high ornamental value. In addition, Taiwan lily has certain edible and medicinal value, Run lung and cough, clearing heat and diuresis [3]. Taiwan lily is a very vigorous plant. It was widely distributed in Taiwan. It can be seen from the seashore to the mountain at an altitude of 3,500 meters. However, in recent years, the number of people has been reduced due to human picking and habitat destruction. It is already in danger. Taiwan lily is usually propagated by bulbs. This method is easy to cause problems such as virus accumulation and species degradation, resulting in a decline in the quality of Taiwan lily. Tissue culture can not only rapidly propagate the plants, but also can produce virus-free seedlings from the uninfected tissues near the growth point. Although there are reports of a large number of primary lily and commercial lily tissue culture [4-11], However, there are relatively few reports on the tissue culture of Taiwan lily [12]. Therefore, we used Taiwan lily scales and leaves sampled from different periods as explants to explore the optimal medium for differentiation and subculture. The composition and culture conditions of the small bulbs
of Taiwan lily were studied, and the complete technical system of tissue culture and detoxification of Taiwan lily was formed. The results of our research are reported below.

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2. Experimental materials and methods

2.1. Experimental materials

The test lily of Taiwan was purchased from Guangzhou Flower Market and planted at the flower breeding base of Aba Teachers College.

2.2. Test methods

2.2.1. Explant treatment. (1) Scales

Select the scales of the diseaseless and robust Taiwanese lily, wash it with water, and rinse with running water for 20 minutes. Disinfect with 75% alcohol for 30s, then rinse with sterile water for 4~5 times, then disinfect the Taiwan lily scales with 0.1% mercuric chloride disinfectant for 10min [7], and finally rinse with sterile water for 4~5 times. You can start vaccination.

(2) blade

The young leaves with no disease spots on the stem tip were cut and rinsed for 20 min. Disinfect with 15% alcohol for 15s, then rinse with sterile water for 4~5 times, then disinfect the Taiwan lily scales with 0.1% mercuric chloride disinfectant for 5min [7], and finally rinse with sterile water for 4~5 times. You can start vaccination.

2.2.2. Medium. Using MS as the basic medium, adding 0.6% agar as coagulant, 4% sucrose as carbon source, 50mg/L activated carbon [13], 0.02% mancozeb as bacteriostatic agent, adding different concentration combinations The hormone is then adjusted to a pH of 6.0.

2.2.3. Culture conditions. The culture temperature was 25±2º C., the light intensity was 2000 L×, and the light treatment was performed for 10 hours per day.

2.2.4. Screening of differentiation medium for sampling scales in winter. The sterilized scales were cut into 0.5-1 cm2 size and inoculated into the differentiation-inducing medium. The medium was set on the basis of MS medium, and GA3 (0, 100, 200, 300 mg/L) and NAA (0.1 mg/L) were set. The ratio of 6-BA (0.5, 1 mg/L) was studied to study the effect of different combinations on the induction of adventitious buds in scales.

2.2.5. Screening of induced differentiation medium for winter sampling leaves. The sterilized leaves were cut into 0.5-1 cm2 size and inoculated into the differentiation-inducing medium. The medium was set on the basis of MS medium, and GA3 (0, 100, 200, 300 mg/L), NAA (0.1, 0.2 mg/L) was set. KT (1mg/L) was used to compare the effects of different combinations on the induction of callus and adventitious buds in leaves.

2.2.6. Spring sampling scale differentiation medium screening. The spring sampling Taiwan lily scales were disinfected and inoculated into the medium of MS+NAA (0.1, 0.2, 0.5, 0 mg/L) + 6-BA (0.5, 1.5 mg/L), and the scales were treated as above.

2.2.7. Screening of induced differentiation medium for spring sampling leaves. Orthogonal experiment was used to screen the optimal medium for inducing leaf differentiation of Taiwan lily in spring. The orthogonal design was carried out using IBM SPSS statistics 20 software. The
experimental design of NAA, KT, TBA, and 6-BA 4 factors 3 levels is shown in Table 5, and the treatment of the blades is the same as above.

2.2.8. Callus differentiation of small bulbs. The callus-induced callus was inoculated into the medium of MS+NAA (0.05, 0.1, 0.2 mg/L) + KT (1 mg/L), and the callus differentiated into small bulbs.

2.2.9. Rooting medium screening. Tissue culture-induced small bulbs were inoculated on MS+NAA (0.1, 0.5 mg/L), 6-BA (0, 0.5, 1.5 mg/L) medium to study the optimal medium combination for inducing bulb rooting.

2.2.10. Refining of tissue culture seedlings. When the tissue culture seedling grows to about 3cm, the tissue culture flask can be removed for refining. The refining substrate is a 1:1 ratio of vermiculite plus perlite [1]. The tissue culture seedlings with better rooting are carefully taken out of the tissue culture flask. Carefully wash the root residual medium, then wash the roots of the tissue culture seedlings with 0.1% carbendazim, transfer to the seedling substrate for cultivation, first place indoors or in a cool place for half a month, during which time pay attention to the moisturizing of the substrate, then move to Outdoor half-day sunshine, waiting for it to adapt to the outdoor environment.

3. Results and analysis

3.1. Screening of scale-inducing differentiation medium in winter

In the winter when the lily tissue culture, due to the existence of natural dormancy, explant differentiation rate is very low or even unable to differentiate. At present, the treatment of bulbs with low temperature or gibberellin is usually used to relieve lily dormancy [2,9], we studied The effects of gibberellin on bulb differentiation during the tissue culture of Taiwan lily were carried out in winter. The experimental results are shown in Table 1 and Table 2. After 8 days of inoculation of Taiwan lily, some tissues with gibberellin began to grow white small protrusions. See Figure 1A. After 12 days of inoculation, most of the explants in the culture flask with gibberellin showed green protrusions. A small amount of differentiated small bulbs, as shown in Figure 1B, at this time, only a small amount of explants were observed to differentiate without gibberellin culture conditions. It can be seen from Table 2 in Table 1 that when the Taiwan lily flower scales were sampled as explants to induce adventitious buds in winter, the addition of a certain concentration of gibberellin to induce adventitious buds was better than the case without gibberellin. MS+0.2 ug/L GA3+0.1 mg/L NAA+1 mg/L 6-BA is the optimal scale-differentiating medium. After 10 days of culture, the differentiation rate is 84%, and the differentiation rate reaches 92% after 30 days of culture. The highest differentiation rate was only 12% after 10 days of culture without adding gibberellin, and the highest differentiation rate was 88% after 30 days of culture. From this point of view, the addition of gibberellin has a certain effect on breaking the dormancy of Taiwan lily, but the condition of not adding gibberellin also showed a higher differentiation rate after 30 days. From this, it seems that Taiwan lily is in winter. Not in a full sleep state, but in a semi-sleep state. It can also be seen from Table 2 in Table 1 that when the concentration of gibberellin in the medium reaches 0.3 ug/L, the browning rate of the explants is significantly increased. This concentration level of gibberellin on the scales of Taiwan lily is explanted. The body should have large damage. When the concentration of gibberellin in the medium is less than 0.2 ug/L, there is no significant effect on the browning of explants.

3.2. Screening of induced differentiation medium for winter sampling leaves

When the lily of Taiwan is cultivated outdoors in Sichuan, it will germinate again in autumn after flowering withered in summer. In winter, it will stop growing due to low temperature. In winter, Taiwan lily leaves are taken as explants for tissue culture. After 120 days of culture, the results are shown in Table 3. The Taiwan lily flower samples taken in winter are difficult to differentiate into
adventitious buds or callus. From Table 3, it can be seen that there is a higher rate of browning necrosis under each culture condition. After one week of inoculation, most of the leaves begin to gradually necrosis. After 30 days of culture, no adventitious buds or callus were observed, and a small amount of adventitious buds or callus were differentiated after 100 days of culture (see Fig. 1E and Fig. 1F), wherein MS+0.1 ug/L GA3+0.1 mg/L NAA +1 mg/L 6-BA was the optimal leaf differentiation medium under this condition. The adventitious bud differentiation rate was 8%, and the callus induction rate was 18%.

Table 1. Statistics on growth of Taiwan lily flower scales after 10 days of winter sampling (mg/L)

| Serial number | GA3  | NAA  | 6-BA | Number of vaccinations | Germination number | Browning number | Germination rate | Browning rate |
|---------------|------|------|------|------------------------|--------------------|-----------------|-----------------|--------------|
| A1            | 0    | 0.1  | 1    | 50                     | 6                  | 0               | 0.12            | 0.00         |
| A2            | 0.1  | 0.5  | 50   | 5                      | 2                  | 0.10            | 0.04            |
| A3            | 100  | 0.1  | 1    | 50                     | 36                 | 0.72            | 0.00            |
| A4            | 0.1  | 0.5  | 50   | 18                     | 0                  | 0.36            | 0.00            |
| A5            | 0.1  | 1    | 50   | 42                     | 0                  | 0.84            | 0.00            |
| A6            | 0.1  | 0.5  | 50   | 30                     | 2                  | 0.60            | 0.04            |
| A7            | 0.1  | 1    | 50   | 29                     | 6                  | 0.58            | 0.12            |
| A8            | 0.1  | 0.5  | 50   | 28                     | 2                  | 0.56            | 0.04            |

Table 2. Statistics on growth of Taiwan lily flower scales after 30 days in winter (mg/L)

| Serial number | GA3  | NAA  | 6-BA | Number of vaccinations | Germination number | Number of necrosis | Germination rate | Necrosis rate |
|---------------|------|------|------|------------------------|--------------------|--------------------|-----------------|--------------|
| B1            | 0    | 0.1  | 1    | 50                     | 44                 | 0                  | 0.88            | 0            |
| B2            | 0.1  | 0.5  | 50   | 40                     | 2                  | 0.80              | 0.04            |
| B3            | 0.1  | 1    | 50   | 42                     | 0                  | 0.84              | 0              |
| B4            | 0.1  | 0.5  | 50   | 37                     | 0                  | 0.74              | 0              |
| B5            | 0.1  | 1    | 50   | 46                     | 0                  | 0.92              | 0              |
| B6            | 0.1  | 0.5  | 50   | 40                     | 2                  | 0.80              | 0.04            |
| B7            | 0.1  | 1    | 50   | 29                     | 10                 | 0.58              | 0.20            |
| B8            | 0.1  | 0.5  | 50   | 39                     | 7                  | 0.78              | 0.14            |

Table 3. Statistics on growth of Taiwan lily flower leaves after 120 days of winter sampling (mg/L)

| Serial number | GA3  | NAA  | KT  | Number of vaccinations | Callus number | Germination number | Number of necrosis | Call injury rate | Germination rate | Necrosis rate |
|---------------|------|------|-----|------------------------|---------------|--------------------|--------------------|------------------|-----------------|--------------|
| C1            | 0    | 0.1  | 1   | 50                     | 4             | 0                  | 17                 | 0.08             | 0.00            | 0.34         |
| C2            | 0.2  | 1    | 50   | 0                      | 0             | 15                 | 0.00               | 0.00             | 0.00            | 0.30         |
| C3            | 100  | 0.1  | 1   | 50                     | 9             | 4                  | 20                 | 0.18             | 0.08            | 0.40         |
| C4            | 0.2  | 1    | 50   | 6                      | 0             | 33                 | 0.12               | 0.00             | 0.00            | 0.66         |
| C5            | 200  | 0.1  | 1   | 50                     | 0             | 40                 | 0.00               | 0.00             | 0.00            | 0.80         |
| C6            | 0.2  | 1    | 50   | 2                      | 0             | 24                 | 0.04               | 0.00             | 0.00            | 0.48         |
| C7            | 300  | 0.1  | 1   | 50                     | 4             | 0                  | 17                 | 0.08             | 0.00            | 0.34         |
| C8            | 0.2  | 1    | 50   | 2                      | 0             | 6                  | 0.04               | 0.00             | 0.00            | 0.12         |
3.3. Screening of scale-inducing differentiation medium in spring

In the spring sampling, the scales were used as explants for tissue culture. The scales showed signs of differentiation after inoculation for about 7 days. Small bulbs were produced after 15 days of culture (Fig. 2A). The growth results after 30 days of culture are shown in Table 4. The scaled explants sampled in spring were easy to differentiate into adventitious buds, and MS+0.2 mg/L NAA+1.5 mg/L 6-BA was the best condition for differentiation. The value-added coefficient was 6.73, which averaged 6.73 for each explant. An adventitious bud.

| Serial number | NAA Number of vaccinations | Number of vaccinations | Induction number | Differentiation number | Induction rate | Differentiation coefficient |
|---------------|---------------------------|------------------------|------------------|------------------------|----------------|-----------------------------|
| 1             | 0.0                       | 0.5                    | 50               | 45                     | 156            | 0.90                        | 3.47                        |
| 2             | 0.1                       | 0.5                    | 50               | 48                     | 125            | 0.95                        | 2.50                        |
| 3             | 0.1                       | 1.5                    | 50               | 50                     | 185            | 1.00                        | 3.70                        |
| 4             | 0.2                       | 0.5                    | 50               | 45                     | 128            | 0.90                        | 2.56                        |
| 5             | 0.2                       | 1.5                    | 50               | 45                     | 303            | 0.90                        | 6.73                        |
| 6             | 0.5                       | 0.5                    | 50               | 50                     | 108            | 1.00                        | 2.16                        |
| 7             | 0.5                       | 1.5                    | 50               | 48                     | 105            | 0.95                        | 2.10                        |

3.4. Screening of induced differentiation medium for spring sampling leaves

In the spring, the leaves of Taiwan lily were sampled as explants for tissue culture. After 30 days of culture, some explants began to differentiate (see Figure 2B), compared with winter sampling leaves (including gibberellin and no gibberellin added). The time when the callus appeared to be shorter was much shorter. From this, it can be seen that the leaves of Taiwan lily are dormant in winter, and the addition of gibberellin is not significant for breaking the leaf dormancy. A certain proportion of leaf explants will be browned and necrotic during the cultivation process, and the growth results after 60 days of culture are shown in Table 5. It can be seen from Table 5 that when KT is 1 mg/L, TBA is 0.5
mg/L, and 6-BA is 0.5 mg/L, the differentiation effect is better, and the differentiation differentiation rate is 36%. Compared with scale explants, the differentiation of Taiwan lily leaves is very difficult. The factors may be complex. The leaf tissue is thinner than the scale tissue, and the damage of the explants is relatively large. One of the reasons is that Li Xueyan [4] et al. studied the tissue culture of oriental lily flower leaves, indicating that the sterile seedlings induced by tissue culture were explants, and the leaves began to expand after about 14 days of culture, and about 28 days. The callus has a high differentiation coefficient.

Table 5. Spring sampling, growth statistics (mg/L) after 60 days of Taiwan leaf culture

| NAA | KT  | TBA  | 6-BA | Number of vaccinations | Necrosis | Induction | Necrosis rate | Induction rate |
|-----|-----|------|------|-------------------------|----------|-----------|---------------|----------------|
| 0   | 0   | 0    | 0    | 50                      | 10       | 0         | 0.20          | 0.00           |
| 0   | 1   | 0.5  | 0.5  | 50                      | 4        | 18        | 0.08          | 0.36           |
| 0   | 2   | 1    | 1    | 50                      | 5        | 13        | 0.10          | 0.26           |
| 0.25| 0   | 0.5  | 1    | 50                      | 4        | 7         | 0.08          | 0.14           |
| 0.25| 1   | 1    | 0    | 50                      | 8        | 9         | 0.16          | 0.18           |
| 0.25| 2   | 0    | 0.5  | 50                      | 14       | 5         | 0.28          | 0.10           |
| 0.5 | 0   | 1    | 0.5  | 50                      | 7        | 8         | 0.14          | 0.16           |
| 0.5 | 1   | 0    | 1    | 50                      | 7        | 9         | 0.14          | 0.18           |
| 0.5 | 2   | 0.5  | 0    | 50                      | 5        | 4         | 0.10          | 0.08           |

Figure 2. Growth and rooting culture of Taiwan lily planted in spring

A. Scale culture for 15 days; B. Leaf culture for 30 days; C. Leaf culture for 45 days; D. Leaf culture for 60 days; E. Callus differentiated with small shoots (proliferation culture for 6 days); Rooting culture for 30 days.

3.5. proliferation medium screening

After the callus was transferred to the proliferation medium, part of the callus was observed to differentiate into small bud spots on the 6th day (see Table 2E), but then the growth was slower. After 30 days, the statistical results are shown in Table 6. MS+0.2 mg/L NAA+1.0 mg/L KT was the best proliferation medium, the differentiation rate was 90%, and the proliferation coefficient was 2.61.
Table 6. Statistics on growth after 30 days of proliferation culture (mg/L)

| Serial number | NAA (mg/L) | KT | Number of vaccinations | Differentiation number | Differentiation rate | Value-added coefficient |
|---------------|------------|----|------------------------|------------------------|---------------------|------------------------|
| H1            | 0.2        | 1  | 20                     | 18                     | 0.90                | 2.61                   |
| H2            | 0.1        | 1  | 20                     | 15                     | 0.75                | 1.87                   |
| H3            | 0.05       | 1  | 20                     | 13                     | 0.65                | 1.39                   |

3.6. Rooting medium screening

The small bulbs produced on the induced differentiation medium were inoculated to the rooting medium, and the roots began to differentiate after various culture conditions for about 10 days. The statistical results are shown in Table 7 after 30 days of culture. From the table, it can be seen that the culture condition with NAA of 0.5 mg/L is more than the culture condition of NAMA of 0.1 mg/L, and the root is also more robust; MS+0.5 mg/L NAA+0.5 mg/L 6-BA is the best induction rooting medium, at which time the roots are well developed and the strongest, although the number of roots produced under MS+0.5 mg/L NAA is the most, but it is relatively weak.

Table 7. Statistics on growth after 30 days of rooting culture (mg/L)

| NA A 6-BA | Number of vaccinations | Induction number | Induction rate | Number of roots | Growth |
|-----------|------------------------|------------------|----------------|----------------|--------|
| 0.1 0     | 20                     | 18               | 0.90           | 51             | A few have root hair and the root is thinner |
| 0.1 0.5   | 20                     | 20               | 1.00           | 37             | A few root hairs with fine roots |
| 0.1 1.5   | 20                     | 15               | 0.75           | 26             | A few root hairs, and the roots are thinner |
| 0.5 0     | 20                     | 19               | 0.95           | 61             | Most have root hair, root slender |
| 0.5 0.5   | 20                     | 19               | 0.95           | 40             | Root hair, thick root |
| 0.5 1.5   | 20                     | 20               | 1.00           | 44             | With root hair, partially rooted |

3.7. Refining and transplanting

The robust rooting seedlings were removed from the tissue culture bottle and reconstituted. The transplanting time was mid-April. The local outdoor temperature during the day was about 20 °C. After 30 days of transplanting, the statistical survival rate was 100%, indicating that our Taiwanese lily The transplanting method of tissue culture seedlings is effective, and Taiwan lily also shows better adaptability to the local environment.

4. Discussion and outlook

As mentioned above, Taiwan lily has better ornamental value and certain medicinal edible value, so it is necessary to study the rapid propagation technology system of Taiwan lily. Tissue culture is the main method to achieve detoxification and rapid propagation of plants. In theory, all parts of plants can be used as explants for tissue culture, but different tissues and organs have different characteristics as explants. Plant tissues with strong convenience and differentiation ability often use scales, leaves and seeds as explants in the tissue culture of various lilies. In this study, we studied the rapid propagation of Taiwan lily flower tissue by using Taiwan lily scales and leaves sampled in different seasons. The results showed that the optimal medium for sampling Taiwan lily flower scale differentiation was MS. +0.2 ug/L GA3+0.1 mg/L NAA+1 mg/L 6-BA; the optimum medium for sampling and differentiation of Taiwan lily leaves was MS+0.1 ug/L GA3+0.1 mg/L NAA+1 Mg/L KT; the best medium for differentiation and differentiation of Taiwan lily flower scales was MS+0.1 mg/L NAA+1.0 mg/L 6-BA in spring; the best medium for induction and differentiation of Taiwan lily
flower in spring was MS+0.1 Mg/L NAA+1 mg/L IBA; optimal callus differentiation bulb medium was MS+0.2 mg/L NAA+1.0 mg/L KT; optimal induction rooting medium was MS+0.5 mg/L NAA+0.5 mg/L 6-BA; perlite: 1:1 refining and cultivating substrate is a suitable refining and cultivating substrate.

From the results of the study, it is possible to induce adventitious buds or callus by using young leaves of stems as explants, but it is difficult to induce. In winter, the explants need to be cultured for about 100 days to observe adventitious buds or callus. Differentiation, spring sampling leaves also need to be cultured for about 30 days to observe signs of differentiation, which indicates that Taiwan lily plants have a certain degree of dormancy in winter; while Taiwan lily scales are more likely to induce differentiation of adventitious buds, and spring sampling scales are cultured for about 7 days. That is, differentiation began, and the scales of winter sampling were observed on the 8th day of culture on the medium supplemented with the appropriate amount of gibberellin, and the scale differentiation was observed only a few days later without the addition of gibberellin. Therefore, the rapid development of Taiwan lily flower tissue is suitable for explants with scales, rapid differentiation and large differentiation coefficient; while Taiwan stem end leaves can be used to produce Taiwan lily detoxification seedlings. In addition, when the Taiwan lily flower scales were used as explants, the differentiation was mainly caused by small bulbs or roots, while when Taiwan lily leaves were used as explants, the callus was more easily differentiated, and the reason was further studied.

The research on the tissue culture and rapid propagation technology system of Taiwan lily is not only beneficial to the commercial development of Taiwan lily and the breeding of new lily varieties, but also can effectively protect the wild resources of Taiwan lily and reduce the artificial picking damage. At present, our research on the rapid propagation technology system of Taiwan lily is only preliminary. Next, we will also explore the differentiation of stems, periments and anthers of Taiwan lily, as well as the sterile cell line of Taiwan lily. Plant regeneration was established and passed through suspension cell lines.

Acknowledgments

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