Tissue Culture and Establishment of Rapid Propagation System of Valeriana Jatamansi Jones

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Abstract

In order to study the rapid and complex system of Valeriana jatamansi Jones (Valeriana wallichii DC.), it provides reference data for the factory cultivation. Tissue cultural and rapid propagation techniques for Valeriana jatamansi Jones were studied through rhizome buds, leaves, stalksas explants. The optimum conditions were selected through the ratio of different hormone concentrations. It turned out that rhizome bud of Valeriana jatamansi Jones was the most suitable explants. Valeriana jatamansi Jones was a perennial herb, its dry rhizome and root can be medicine, currently wild resources were deficient. Tissue cultural and rapid propagation techniques for Valeriana jatamansi Jones were studied through rhizome buds, leaves, stalksas explants. It turned out that rhizome bud of Valeriana jatamansi Jones was the most suitable explants. The suitable callus induction medium was MS medium containing 3.0 mg·L^{-1} 6-BA and0.1 mg·L^{-1} 2, 4-D, the induction rate was 88.7%; the suitable proliferation medium was MS medium containing 3.0 mg·L^{-1} 6-BA and 0.3 mg·L^{-1} NAA, the proliferation rate was 93.3%;the suitable rooting medium was 1/2MS medium containing 1.5 mg·L^{-1} NAA, the rooting rate was 100%.

Keywords— *Valeriana jatamansi* Jones; Explants; Tissue cultural; Rapid propagation system

I. INTRODUCTION

Alias of *Valeriana jatamansi* Jones (*Valeriana wallichii* DC.) are Saruma henryi and others, *Valeriana jatamansi* Jones. is a kind of herbaceous perennial in Valerians of Valerianaceae, Its dry rhizomes and roots can be used as medicine [1-3]. Plant height is 20 ~ 70cm. Rhizome has a few branches, basal leaves developed covered by pubescent or glabrous crust. Rhizome is massive, thick and hypertrophy with tight internodes and petiole residues in specific aroma. *Valeriana jatamansi* Jones. usually grow in the valley under the damp forest or slopes wet, meadow of peaks , open land in forest edge and roadside tussock, and mainly distributed in China's Shanxi, Chongqing, Henan, Gansu, Sichuan, Guangxi and other regions[4-5]. *Compendium of Materia Medica* had documented: *Valeriana jatamansi* Jones, it is grass root grown in Song pan Mountain in the westward of Sichuan. The color is black and it has thick tendril likes spider, sichuan lovage rhizome with fragrance. With the continuous development of medicinal resources of *Valeriana jatamansi* Jones, wild resources and traditional breeding methods have not met the contradiction between market supply and demand. At present, there are no reports of tissue culture in spider culture at home and abroad [6-8]. The research mainly focused on chemical composition, pharmacological action, cultivation introduction, quality standard of medicinal materials and so on. Wang Weiqianstudied the chemical constituents of *Valeriana jatamansi* Jones, and extracted the new component of iridoid ester: valerjatadoid C[9]; Zhang Ningning found that the iridoid of *Valeriana jatamansi* Jones has a significant effect in aspect of anti-tumor, anti-anxiety and treatment of gastrointestinal disease[10]; Zhang Yanping et alstudied the cultivation techniques of *Valeriana jatamansi* Jones.[11]. The study selects wild *Valeriana jatamansi* Jones for tissue culture research,

in order to establish rapid propagation system of *Valeriana jatamansi* Jones, which is of great significance in development of *Valeriana jatamansi* Jones.

II. MATERIALS AND METHODS

A. Test Material

Good and robust wild *Valeriana jatamansi* Jones collected from Kirin District of Qujing City, Yunnan Province.

B. Cultivation Conditions

The experimental culture temperature was 25 ± 1 ℃, the light intensity was 2000 ~ 3000 lx, the illumination time was 14 h / d, and the ambient humidity was 60% ~ 70%.

C. Test methods

1) Medium Composition:

The basic medium was MS + 3% sucrose +0.6% agar, pH 5.6, adding different concentrations and types of plant growth regulators, sterilization time is 30min.

2) Induction and Culture of Callus:

In the clean bench, deal the explants with 75% alcohol for 30s, use sterile water to rinse 3 to 5 times, and then soak fully in 0.1% $HgCl_2$ for 10-15min, use sterile water to rinse it repeatedly from 4 to 6 times to remove HgCl2 with full oscillation, the surface moisture of the explants was dried with sterile filter paper, then inoculate rhizomatous lateral bud(0.5 cm length), petiole (1.0 cm length) and leaves (area 2.0 cm2) on the induction medium (Table 1), 5 samples per bottle, 30 bottles per treatment, 3 replicates, start observation after 7 days, and count the situation of callus induction after 30 days.

Table 1 Callus Induction Effect of Experimental Design

Medium	6-BA（mg·L^{-1}）	2，4-D（mg·L^{-1}）
A1	0.0	0.0
A2	1.0	0.1
A3	1.0	0.3
A4	1.0	0.5
A5	3.0	0.1
A6	3.0	0.3
A7	3.0	0.5
A8	5.0	0.1
A9	5.0	0.3
A10	5.0	0.5

3) Increment Culture of Callus:

Inoculate successful callus on test medium (Table 2), 5 samples per bottle, 30 bottles per treatment, The fresh weight of each bottle was weighed, and re-weighed the culture medium after 45 days, three replicates, collect the situation of callus proliferation, select the optimum medium for callus proliferation of *Valeriana jatamansi* Jones.

Table 2 Callus Increment Effect of Experimental Design

Medium	6-BA（mg·L^{-1}）	NAA（mg·L^{-1}）
B1	0.0	0.0
B2	1.0	0.1
B3	1.0	0.3
B4	1.0	0.5
B5	2.0	0.1
B6	2.0	0.3
B7	2.0	0.5

B8	3.0	0.1
B9	3.0	0.3
B10	3.0	0.5

*4) **Rooting Culture***

Inoculate tissue culture seedling of *Valeriana jatamansi* Jones on 1 / 2MS basic medium + 0.5 ~ 2.0 mg· L-1 IAA. 20 bottles per treatment, 3 replicates and 60 d for one cycle, observe and record the rooting condition of seedlings of *Valeriana jatamansi* Jones, select the optimum medium for seedlings of *Valeriana jatamansi* Jones.

*5) **Transplanting Seedlings***

After 3 to 5 day's gardening-seedlingat room temperature, transplants them to the greenhouse when culture seedlings of *Valeriana jatamansi* Jones grow to 4 ~ 5cm, root length of 3 ~ 4cm. Culture temperature is 25 ± 2 ℃, humidity is 70% ~ 80%. Substrate with perlite and vermiculite by 1 to 1 was the optimal, observes the growth condition, and records the survival rate of transplantation.

D. Data Analysis

Use Excel2007 and SPSS 17.0 software to make a statistical analysis with the test data.

III. RESULTS AND ANALYSIS

A. Callus Induction Analysis in Different Parts of the Explants

The rhizomatous lateral bud of *Valeriana jatamansi* Jones expanded after 6 ~ 8 days, and formed more calli after 30 days, in short time, the adventitious buds were induced in A6 medium and the number of buds was good; The petiole was explored as explants, and the base expanded at about 5 days, the top expanded later, petiole color became from green to light green and formed more callus after 30 days, but it was easy to browning. Leaf culture appeared lens at first in the edge of leaves since 7d, formed a large number of calluses around 23d, and appeared different degrees of browning. As shown in Table 3, the best culture medium for petiole callus induction was A5 and the induction rate was 72.7%. The best medium for leaf callus induction was A7 and the induction rate was 91.3%; the best medium for rhizomatous lateral bud callus induction was A5, the induction rate was 88.7%, which callus growth potential was the best.

Table 3 Effect of the Induction of Callus in Different Medium

Medium	Callus growth condition			Induced callus production/piece			Induction rate/%		
	Rhizomatous lateral bud	petiole	Leaf	Rhizomatous lateral bud	Petiole	Leaf	Rhizomatous lateral bud	Petiole	Leaf
A1	－	－	－	0	0	0	0	0	0
A2	－	－	－	0	0	0	0	0	0
A3	－	＋	－	0	97c	0	0	64.7c	0
A4	＋	＋	－	82b	101b	0	54.7a	67.3b	0
A5	＋＋	＋＋	－	133a	109a	76d	88.7a	72.7a	0
A6	－	－	＋	0	0	110 b	0	0	73.3 b
A7	－	－	＋＋	0	0	137 a	0	0	91.3 a
A8	－	－	－	0	0	0	0	0	0
A9	－	－	+	0	0	89c	0	0	59.3 c

A10	—	—	+	0	0	61e	0	0	40.7 d

B. Analysis of Callus Proliferation Culture

Table 4 Callus Proliferation Culture

Medium	Callus proliferation	Multiplication rate%
B1	Callus gradually bud, few bud, no proliferation	0
B2	Callus gradually bud, few bud, no proliferation	0
B3	Callus gradually bud, less bud, no proliferation	0
B4	Callus gradually bud, less bud, no proliferation	0
B5	Callus growth is slow, gradually bud, less buds	19.3f
B6	Inconspicuous Callus growth, gradually bud and a little adventitious root	23.6e
B7	Inconspicuous Callus growth, gradually bud and a little adventitious root	56.0d
B8	Callus swelling rate is relatively slow, more buds	71.5c
B9	Callus gradually expanded, grow well, more and robust buds	93.3a
B10	Most callus expand, growth is better, more buds	73.7b

After 45d of callus sub inoculation, callus in B1 ~ B4 medium produced less buds and no new callus; Callus in B5, B6, B7, B8 and B10 medium produced new buds around 15 days later and regenerated buds; Callus in B6 ~ B7 medium regenerated many adventitious roots; Callus in B9 medium has best effect in proliferation, which not only can produce rapid callus, but also has the highest number of regenerated buds and growth rate. The proliferation rate is up to 93.3%.

C. Analysis of Rooting Culture

Table 5 Effects of Different Concentrations of NAA on Rooting

Treatment	Concentrations of NAA	Root number	Root length（cm）	Rooting rate（%）
1	0	1.3d	0.65d	78c
2	0.5	2.75c	0.95cd	93b
3	1.0	3.5b	1.20c	100a
4	1.5	4.1ab	2.57a	100a
5	2.0	4.7a	1.85b	100a

From Table 5, the root number of *Valeriana jatamansi* Jones tube seedling, root length and rooting rate increased with the increase of NAA concentration in the culture medium, the root number will increased but more easlier in callus when the concentration was more than 1.5 $mg \cdot L^{-1}$. Therefore, rooting culture in 1 / 2MS + NAA1.5 $mg \cdot L^{-1}$. root grow n near the veins, so the number of roots was higher, the root growth was normal, rooting rate was 100%.

D. Transplanting Tempering Seedings

According to the test method, the survival rate of tissue culture seedlings of *Valeriana jatamansi* Jones that were transplanted successfully after hardening can be up to more than 95%. They grown new leaves and roots after 20days with a well growth and few lesions, achieved the desired results.

IV. DISCUSSION

The study established the rapid propagation system of *Valeriana jatamansi* Jones for tissue culture studies consist of different explants and different medium. The results were as follows: the best explant of callus induction was rhizomatous lateral bud, and best formulation is MS + 6-BA 3.0 $mg{\cdot}L^{-1}$ + 2,4-D 0.1 $mg{\cdot}L^{-1}$, it is similar to study results for Asarum henryi of Li Huining's research, the proportion of plant auxin and cell taxin was same, and the callus induction rate was higher. The callus culture was MS + 6-BA 3.0 $mg{\cdot}L^{-1}$ + NAA 0.3 $mg{\cdot}L^{-1}$, and the rooting culture was 1 / 2MS + NAA 1.5 $mg{\cdot}L^{-1}$, which was differ from Metka Šiško' tissue culture which study on *Hydrangea macrophylla* that rooting rate can be 100% with a 0.5 $mg{\cdot}L^{-1}$ concentration of NAA [12]. After exercising of tissue culture seedlings of *Valeriana jatamansi* Jones, they can grow well and rapidly without lesion and insect pests in ventilated greenhouse, and also maintain its superior parent character.

V. CONCLUSION

The basic medium is the basic substrate of plant tissue culture. Water, organic matter, nitrogen source and carbon source and the necessary conditions of growth can be provided for explants by MS medium that as broad spectrum medium. Plant growth regulators are less used in the process of plant tissue culture, but the type and proportion concentration are also the key in influencing plant tissue culture. The rhizomatous lateral bud of *Valeriana jatamansi* Jones that were used as explants can induce a large number of callus in a short time, and a large number of adventitious buds to cultivate root. The induction medium was MS + 6-BA 3.0 $mg{\cdot}L^{-1}$ + 2, 4-D 0.1 $mg{\cdot}L^{-1}$, its induction rate was 88.7%; The proliferation medium was MS + 6-BA 3.0$mg{\cdot}L^{-1}$ + NAA 0.3 $mg{\cdot}L^{-1}$, the proliferation rate was 93.3%; The rooting medium was 1 / 2MS + NAA 1.5 $mg{\cdot}L^{-1}$, and the rooting rate was up to 100%. tissue culture seedlings of *Valeriana jatamansi* Jones can obtain superior seedlings after acclimatization and transplanting, and significant basis was provided by its rapid propagation system for the industry of *Valeriana jatamansi* Jones.

VI. CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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