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Ri-gene transformation into leaf tissues of Artemisia annua L. and cultivation of hairy-root for artemisinin accumulation

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***Abstract*—The medium B5 was favored for root initiation. Strong initiation of root was leaves used as materials supplemented with NAA (0.5 mg/l). Cytokinin (BA, kinetin) and IAA, IBA were not favored for root initiation cultures. Leaves of in vitro plantlets were used as materials for gene transformation. The method of agrobacterium for gene transformation was effectiveness. To obtain the leaves having gene transformation and release agrobacterium. Hairy-roots were established on medium of B5 + NAA (0.5 mg/l) and used as materials for liquid culture research. The modified MS liquid-media having sucrose (50 g/l), regulating of $NH4^+/NO3^-$ (5/25), supplemented with NAA (0.5 mg/l) enhance artemisinin accumulation up to 1.468 %.**

***Keywords*—Artemisia annua L., Ri-gene, gene transformation, hairy-root, accumulation**

I. Introduction

Artemisinin is a potent antimalarials nowadays, but artemisinin biosynthesis is still under study and low levels of naturally occurring exotic plants are extracted from *Artemisia Annu* L. of 0.01% -0.5% relative to dry weight and high content when flowering is just blossoming at flowering stage [1]. The artemisinin biosynthesis is difficult due to its complex structure, depending on the variety, cultivation conditions and geography. Chlomequat has been used to raise artemisinin levels but there is no effective way to provide adequate artemisinin to the 490 million people who are sick and block millions of deaths annually.

Culture tissue plays an important role in gene transfer. Many transgenic studies on tissue culture of *Artemisia Annu* L. usually use young culture material. However, there are also many studies using cell suspension. In root culture studies, root-forming and non-callus tissue forms, resulting in high root growth efficiency in liquid culture on a shaker or bioreactor [2].

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In order to obtain high yielding root crops, improved strains begin with the choice of parent plants that the desired product is high in. The different strains of *agrobaterium rhizogenes* may also affect gene transfer. Giri & Narasu [3] showed the contrast of A4, 15834, K599, LBA 9402, and 9340 strains of *A. Rhizogenes*, of which LBA 9365 was found to induce *A. Annua* with higher artemisinin content. However, some other plant species may respond better to other strains of *A. Rhizogenes*. Successful gene transfer to callus tissue regeneration shoots [4], suspension [5] and roots [6, 7, 8] have resulted in increased levels of artemisinin synthesized in the cell 1-5 times.

As hair- root of *Artemisia Annu* L. can accumulate artemisinin, there are many studies on nutrient environment, growth regulator, protein, sugar and light that affect artemisinin synthesis through haity-root culture on liquid medium [2, 9, 10, 11, 12, 13]. In this paper, we study the transformation of hairy-root gene into leaf tissue and influence of liquid culture medium on accumulation of artemisinin content.

II. Material and Methods

A. *Selecting Transgenic Root Tissue*

1) Material

Artemisia Annu L. bean TC1.8 selected lines were sown on MS medium in vitro. Tissue culture is used as a culture material. Leaves, stems and callus tissue are used for root growth.

2) The culture medium

B5 [1], MS [8], LV [6] supplemented with: Kinetin (furfuryl aminopurine), BA (benzylaminopurine), IAA (β-indoleacetic acid), IBA (β-indolebutyric acid), NAA (α-naphthaleneacetic acid), 2,4D (di-chlorophenoxyacetic acid).

3) Conditions of culture

Temperature 28 + 2^{o}C, RH = 65%, Light intensity 22.2 mmol/m^2/s, lighting time 10 hours/day.

4) Method

The experiment was arranged randomly, three times, three times with 3 triangles, each with 7 leaves. Data were recorded after 30 days of culture and analyzed by MSTATC statistical program (p = 0.05).

5) Experimental design

Root formation medium: Use three mineral media MS, B5, LV supplemented with growth regulators of auxin and cytokinin: On semi-solid medium B5 supplemented with BA, Kinetin, IAA, IBA, NAA or 2,4D concentrations (0, 0.1, 0.5, 0.7, 1 mg/l).

Effect of culture tissue: Explants culture (stem 10 mm), leaves (1x1 cm), callus tissue. The semi-solid medium for root formation cultured with B5 supplemented with IAA, IBA and NAA concentrations of 0.1-0.5 mg/l.

B. Transferring the Hairy-Root Gene into Leaf Tissue of Artemisia Annu L.

1) Material

Agrobaterium ATCC 11325 imported from the United States (ITS company). The seedlings of line TC1.8 were obtained from the HCMC selection experiment. TC1 grains were supplied by Hanoi research center for medicinal materials.

Agrobacterium strain cultured on LB medium (Luria Bertani): Tryton 10 g/l, 5 g/l yeast extract, NaCl 10 g/l, PH 7.2. To propagate the gene transfer, the bacteria were shaken overnight in an LB medium at 26 °C.

Sow seeds in vitro was that the experiment was conducted in a process that included: Seeds were washed through the savon for 3-5 minutes. Wear the washed bead on the sterile incubator. Wash with sterile distilled water 2-3 times. Shake in 70% ethanol for 1 minute, rinse with sterile distilled water 3 to 5 times. Soak in hypochlorite solution for 30 minutes. Rinse the sterile solution with sterile distilled water 3-5 times. Place sterilized seeds in 250 ml Erlene containing 50 ml of base MS medium. One month after seed germination will be transferred to MS medium without plant growth regulator.

Genetic transformation process: The fact that artemisinin is synthesized and stored almost exclusively in leaves of *Artemisia Annu* L. , the leaves are used as a source of material for hairy-root gene transfer to produce artemisinin. The mature leaves from in vitro seedlings were cut to 0.3 x 0.3 cm for wound formation. Inoculation of leaf tissue with bacteria for 30 minutes. Gradually remove the excess bacteria from the filter paper. Culture of leaf pieces with bacteria in B5 supplemented with acetosyringone (AS) 100 µm for 2 days. Rinse the bacteria with sterile distilled water. Dissolve the sample, drain and cultured the leaf pieces on B5 medium supplemented with 500 mg/l Cefotaxime to kill the remaining bacteria. Culturing time of 3 weeks was changed to new medium. Repeat 3 times. Observe root formation from transformed leaf tissue samples. Evaluation of the ability to remove *Agrobacterium* by dry root powder ELISA.

2) Design experiment

Establishment of roots in transgenic leaves: Transgenic plants were cultured on B5, MS, LV regenerated roots supplemented with NAA growth regulators (0, 0.1, 0.5, 0.7, 1 mg/l). Tracking indicator is the number of roots formed on cultured specimens after 30 culture days.

Growth of transgenic roots: The roots of 10-30 mm length were cultured in B5 + NAA medium (0.5 mg/l).

C. Culture of Transgenic Hairy-Root on Liquid Medium

1) Material

Artemisia Annu L. grain line TC1.8 line selected

MS, B5, LV medium supplemented with NAA (0.5 mg /l), BA, GA3 (Gibberellic Acid)

2) Conditions of culture

Temperature 28 + 2^{o}C, RH = 65%, light intensity 22.2 µmol/m^{2}/s, lighting time 10 hours/day.

3) Method

The experiment was arranged randomly, three times each, three times with flasks (50 ml culture medium), each with a flask implanted with 100 mg root sample. Data were collected after 21 culture days and analyzed by MSTATC statistical program (p = 0.05).

4) Design experiment

a) Effects of nutrient medium on artemisinin accumulation

Hairy-root was cultured in two B5 and MS mediums, supplemented with NAA (0.5 mg/l).

b) Effect of sucrose on artemisinin accumulation

Hairy-root was cultured on MS + NAA medium (0.5 mg/l) supplemented with sucrose (0, 10, 20, 30, 40, 50, 60, 70, 80, 90 g/l).

c) Effect of nitrate nitrogen on artemisinin accumulation

The hairy-root cultured on MS + NAA (0.5 mg/l) + sucrose (50 g/l) supplemented with nitrate nitrogen (10, 20, 30, 40, 50, 60, 70, 80, 90 mM).

d) Effect of $NH4^{+}/NO3^{-}$ on artemisinin accumulation

The hairy-root grown on MS medium + NAA (0.5 mg/l) + sucrose (50 g/l) $NH4^{+}/NO3^{-}$ (0/30, 5/25, 10/20, 15/15, 20/10, 25/5, 30/0).

e) Effect of inorganic phosphate on artemisinin accumulation

The hairy-root was cultured on MS + NAA medium (0.5 mg/l) + sucrose (50 g/l) supplemented with KH_2PO_4 (0.5-1, 0-1, 5-2, 0-2, 5-3, 0-3, 5 mm).

f) Effect of BA on artemisinin accumulation

The hairy-root cultured on MS + NAA medium (0.5 mg/l) + sucrose (50 g/l) supplemented with BA (0-0, 1-0, 3-0, 5-0, 7-1, 0 mg/l).

g) Effect of GA3 on artemisinin accumulation

The hairy-root was cultured in MS + NAA medium (0.5 mg/l) + sucrose (50 g/l supplemented with GA3 (0, 1, 2, 3, 4, 5, 6, 7 mg/l).

h) Modified medium

The hairy-root was cultured in MS and modified MS medium supplemented with NAA (0.5 mg/l), $NH4^+/NO3^-$ (5/25), sucrose 50 g/l.

g) Culture of *Artemisia Annu* L. hairy-root on Temporary Immersion Bioreactor

Growth dynamics of the roots on temporary immersion system bioreactor: The hairy-root was cultured in a modified MS medium. Time for growth dynamics (0, 5, 10, 15, 20, 25 days after culture) at temporary at floating 1 minutes and sinking 4 hours.

III. Results and Discussion

A. Selecting Tissue Culture for Transgenic Hairy-Root

1) Root formation medium

There are currently three major base medium in labs in the world that are used extensively in rootstock research, MS, B5 and LV. The goal was to choose the right nutrient medium for root regeneration and without the formation of callus tissue in leaves or in newly formed hairy-root. The results showed that B5 medium was rooted and did not form callus tissue from culture leave tissue (Table I).

TABLE I. EFFECT OF BASE MEDIUM ON ROOT REGENERATION

Base medium	IAA (mg/l)		IBA (mg/l)		NAA (mg/l)	
	0.1	0.5	0.1	0.5	0.1	0.5
MS	Creates more roots, less callus tissue	Creates more roots, less callus tissue	Creates more roots, less callus tissue	Creates more roots, less callus tissue	Creates more roots, less callus tissue	Creates more roots, less callus tissue
B5	Create many roots	Create many roots	Create many roots	Create many roots	Create many roots	Create many roots
LV	Create many roots, many callus tissue	Create many roots, many callus tissue	Create many roots, many callus tissue	Create many roots, many callus tissue	Create many roots, many callus tissue	Create many roots, many callus tissue

2) Growth regulators auxin and cytokinin

Cytokinin (BA, Kinetin) was not affect root regeneration, yellowing and non-callus tissue formation. Auxins (IAA, IBA, NAA, 2,4D) play an important role in the formation of roots, which arise in most concentrations. The control was not develop root formation, after 7 days the tissues turn yellowish. Of which NAA (0.5 mg/l) gave more root regeneration than the other auxin (Table II).

TABLE II. EFFECT OF CYTOKININ AND AUXIN ON THE ABILITY OF CULTURED CALLUS TISSUE REGENERATION

Concentration (mg/l)	0	0.1	0.5	0.7	1
BA	Not responding	Yellow, wilt, not create callus tissue	Yellow, wilt, not create callus tissue	Yellow, wilt, not create callus tissue	Yellow, wilt, not create callus tissue
Kinetin	Not responding	Yellow, wilt, not create callus tissue	Yellow, wilt, not create callus tissue	Yellow, wilt, not create callus tissue	Yellow, wilt, not create callus tissue
IAA	Not responding	Create many roots	Create many roots	Create many roots	Create many roots
IBA	Not responding	Create many roots	Create many roots	Create many roots	Create many roots
NAA	Not responding	Create many roots	Create many roots	Create many roots	Create many roots
2,4D	Not responding	Create many roots	Create many roots	Create many roots	Create many roots

3) Effect of culture tissue

Callus and root formation from leave in auxin and culture concentration, strong root formation in leaves with NAA concentrations (0.5 mg/l) (Table III).

TABLE III. EFFECT OF TISSUE CULTURE ON ROOT REGENERATION

Base medium	IAA (mg/l)		IBA (mg/l)		NAA (mg/l)	
	0.1	0.5	0.1	0.5	0.1	0.5
Leave (0,3 x 0,3 cm)	Create many roots	Create many roots	Create many roots	Create many roots	Create many roots	Create many roots
Stem (10 mm)	Create roots at two cuts, less roots	Create roots at two cuts	less roots	Create roots at two cuts	less roots	Create roots at two cuts
Callus (Φ 5 mm)	Create medium roots	Create medium roots	Create medium roots	Create medium roots	Create medium roots	Create medium roots

B. *Transferring the Hairy-Root Gene into Leaf Tissue of Artemisia Annu L.*

1) Regeneration of roots in transgenic leaves

All three MS, B5, LV media were suitable for root regeneration. B5 + NAA (0.5 mg/l) was used in B5 + medium (10.2 roots/culture sample) and did not form callus tissue. On MS and LV media, root regeneration was associated with callus tissue formation (Table IV).

TABLE IV. REGENERATING ROOTS (NUMBER OF ROOTS/CULTURE SAMPLE) IN VITRO

NAA	MS(*)	B5	LV(*)
0.0	3.2	4.6	1.4
0.1	4.2	5.8	2.8
0.5	9.6	10.2	6.8
0.7	6.2	9.8	4.2
1.0	4.5	8.2	3.4
CV(%)	14.2	12.6	16.4

(*): Create roots and callus tissue

2) Growth of transgenic roots

On B5 + NAA medium (0.5 mg/l), the root growth unit (RGU) reached the highest (3.25) on day 14 and subsequently decreased due to the development of the number of hairy-root (7.4) faster growth of main root length (9.8 cm) at day 28 (Table V).

TABLE V. GROWTH OF HAIRY-ROOT

Day after culture	7	14	21	28
Main root length (cm)	1.20	4.50	7.60	9.80
Number of hairy-roots (number of roots)	1.10	3.20	5.50	7.40
Number of hairy-roots per cm	0,.91	0.71	0.72	0.75
Hairy-roots length (cm)	0.12	0.44	0.75	0.92
Total hairy-roots length (cm)	1.30	5.90	4.10	6.80
RGU root growth unit (cm)	2.74	3.25	2.12	2.24

RGU (Root Growth Unit) = [Σ a root length of hairy-root + main root length]/number of hairy-roots (McCoy, 2003)

C. *Grow Hairy-Roots of Artemisia Annua L. on Liquid Medium*

1) Effects of nutrient medium on artemisinin accumulation

Fresh root weight was not different in two basic MS and B5 medium; and there was no callus tissue formation. Root culture on semi-solid medium, MS medium with callus tissue. The MS + NAA medium (0.5 mg/l) was used for liquid culture studies with a concentration accumulation of 1.225% (Table IV) (Figure 1).

TABLE VI. EFFECT OF NUTRIENT MEDIUM ON THE ACCUMULATION OF ARTEMISININ

Base medium	Dry weight of roots (g/l)	Art content (%) of root dry weight
B5	24.5ab	1.146
MS	26.2a	1.225
LV	22.6b	1.086
CV(%)	12.6	

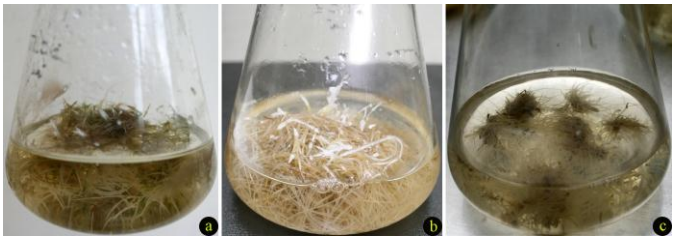

Figure 1. Root growth in the medium of (**a)** B5 (**b)** MS (**c)** LV

2) Effects of sucrose on artemisinin accumulation

Sugar concentration increased by 10-50 g/l, artemisinin accumulation increased by 0.750-1.178%; sugar concentration increased 50-90 g/l, the accumulation capacity increased not significantly. Sucrose concentrations increased by more than 70 g/l, with the possibility of accumulation decreasing. The appropriate culture medium for MS + NAA (0.5 mg/l) + sucrose (50 g/l) had an accumulation of artemisinin content of 1.178% (Table VII).

TABLE VII. EFFECTS OF SUCROSE ON ARTEMISININ ACCUMULATION

Sucrose (g/l)	Dry weight of roots (g/l)	Art content (%) of root dry weight
10	4.5	0.750
20	6.8	0.902
30	12.1	1.089
40	14.5	1.146
50	19.2	1.178
60	22.5	1.182
70	26.2	1.196
80	22.8	1.202
90	20.5	1.212
CV (%)	18.4	

3) Effects of nitrate nitrogen on artemisinin accumulation

Appropriate nitrate concentrations of 30-60 mm, reaching a maximum of 30 mm for 1.198% artemisinin accumulation (Table VIII), have not improved significantly artemisinin accumulation

TABLE VIII. EFFECT OF NITRATE NITROGEN ON ARTEMISININ ACCUMULATION

Nitrate nitrogen (mM)	Dry weight of roots (g/l)	Art content (%) of root dry weight
10	15.5	0.850
20	22.1	0.860
30	25.5	1.198
40	26.2	1.188
50	25.1	1.180
60	25.2	1.150
70	20.5	1.040
80	17.5	0.780
90	13.2	0.650
CV (%)	16.2	

4) Effect of $NH4^{+}/NO3^{-}$ on artemisinin accumulation

Appropriate $NH4^{+}/NO3^{-}$ ratio of 5/25 for 1.386% accumulation; This was higher than artemisinin concentration (Table IX). Significant improvement of artemisinin accumulation compared with nitrogen fixation (30 mm).

TABLE IX. EFFECT OF $NH4^{+}/NO3^{-}$ ON ARTEMISININ ACCUMULATION

$NH4^{+}/NO3^{-}$	Dry weight of roots (g/l)	Art content (%) of root dry weight
0/30	27.2	1.315
5/25	28.4	1.386
10/20	25.4	1.246
15/15	16.8	0.984
20/10	10.5	0.910
25/5	6.6	0.816
30/0	1.5	0.714
CV (%)	12.6	

5) Effect of inorganic phosphate on artemisinin accumulation

Appropriate inorganic phosphate concentration of 1.5 mm with artemisinin accumulation of 1.316%; inorganic phosphate concentrations were higher, and artemisinin depositional capacity decreases (Table X). Inorganic phosphate has not significantly improved the accumulation of artemisinin compared to $NH4^{+}/NO3^{-}$(5/25).

TABLE X: EFFECTS OF PHOSPHATE ON ARTEMISININ ACCUMULATION

KH_2PO_4 (mM)	Dry weight of roots (g/l)	Art content (%) of root dry weight
0.5	21.5	1.288
1.0	25.0	1.298
1.5	23.2	1.316
2.0	30.2	1.052
2.5	30.4	0.754
3.0	30.8	0.746
3.5	31.5	0.720
CV (%)	14.2	

6) Effect of BA on artemisinin accumulation

Appropriate BA concentration was 0.5 mg/l for artemisinin accumulation of 1.364%; higher BA levels, decreased artemisinin accumulation (Table XI). Addition of BA (0.5 mg/l) was not significantly different from that of $NH4^{+}/NO3^{-}$ (5/25).

TABLE XI. EFFECT OF BA ON ARTEMISININ ACCUMULATION

BA (mg/l)	Dry weight of roots (g/l)	Art content (%) of root dry weight
0.0	27.5	1.296
0.1	23.4	1.312
0.3	23.0	1.324
0.5	22.5	1.364
0.7	20.2	1.278
1.0	18.5	1.252
CV (%)	14.2	

7) Effect of GA3 on artemisinin accumulation

The appropriate GA3 concentration was 5 mg/l with the highest artemisinin content of 1.303%; higher concentrations of GA3, decreased artemisinin accumulation (Table XII). The GA3 supplementation (5 mg/l) was not significantly different from the $NH4^{+}/NO3^{-}$(5/25).

TABLE XII. EFFECT OF GA3 ON ARTEMISININ ACCUMULATION

BA (mg/l)	Dry weight of roots (g/l)	Art content (%) of root dry weight
0	27.8	1.233
1	29.5	1.240
2	28.6	1.258
3	28.5	1.268
4	28.0	1.286
5	**27.8**	**1.303**
6	27.0	1.288
7	26.8	1.264
CV (%)	14.2	

8) Modified medium

On MS medium supplemented with NAA (0.5 mg/l), sucrose (50 g / l), $NH4^{+}/NO3^{-}$ (5/25) improvement of artemisinin accumulation. Nitrate, inorganic phosphate, BA, and GA3 have not contributed to the improvement of artemisinin accumulation. The modified MS medium

improved the biomass growth (32.6 g/l) and artemisinin accumulation (1.468%) (Table XIII).

TABLE XIII. EFFECT OF MODIFIED MS MEDIUM ON ARTEMISININ ACCUMULATION

Basic medium	Dry weight of roots (g/l)	Art content (%) of root dry weight
MS (not growth regulator)	28.5	1.146
Modified MS	32.6	1.468
CV (%)	10.8	

9) Culture of *Artemisia annua* L. hairy-root in temporary immersion bioreactor

The results of Table XIV show that, on day 20 after culture, artemisinin content reached the highest concentration of 1.775%. In temporary immersion bioreactor, the culture time may be extended to reach the highest root biomass (Figure 2)

TABLE XIV. THE GROWTH DYNAMICS OF HAIR-ROOTS IN TEMPORARY IMMERSION BIOREACTOR

Time (day after culture)	Dry weight of roots (g/l)	Art content (%) of root dry weight
0	0.0	1.025
5	4.5	0.850
10	12.5	1.186
15	19.5	1.568
20	26.5	1.775
25	27.5	1.764

Figure 2. Dynamic of root growth in temporary immersion bioreactor at the dates after cultivation 5 (a), 10 (b), 15 (c), 20 (d) 25 (e) days and f was control

IV. Conclusion

The B5 medium was suitable for root growth on semi-solid media. Strong root regeneration with culture media was found in leaves and supplemented with NAA (0.5 mg/l), BA, kinetin and IAA, IBA were not suitable for root growth. In vitro young leave were used as a transgenic plant material. The agrobacterium method proved to be effective. Agrobacterium-mediated gene transfer was obtained. The hairy-root forms strongly on semi-solid B5 + NAA medium (0.5 mg/l) and was used as a raw material for liquid culture studies. The liquid medium MS was suitable for growing hairy-roots. The modified liquid MS medium corrects $NH4^+/NO3^-$(5/25), sucrose 50 g/l, NAA supplementation (0.5 mg/l), improving artemisinin accumulation 1.468% compare to MS medium (no growth regulator) artemisinin accumulation 1.146%.

References

[1] J. C. Laughlin, "Agricultural production of artemisinin--a review," PubMed (PMID: 8053017), 2001.

[2] N. B. Paniego and A. M. Giulietti, "Artemisinin production by Artemisia annua L.-transformed organ cultures," Enzyme Microb. Technol., vol. 18, pp. 526–530, 1996.

[3] A. Giri and L. Narasu, "Transgenic hairy roots: Recent trends and applications," Biotechnol. Adv., vol. 18, pp. 1–22, 2000.

[4] J. L. Han, H. Wang, H. C. Ye, Y. Liu, Z. Q. Li, Y. Zhang, Y. S. Zhang, F. Yan and G. F. Li, "High efficiency of genetic transformation and regeneration of Artemisia annua L. via Agrobacterium tumefaciens-mediated procedure," Plant Science, vol. 168, pp. 73–80, 2005.

[5] D. Ramakrishnan and W. R. Curtis, "Fluid dynamic studies on plant root cultures for application to bioreactor design" in S. Furusaki and D. D. Ryu, Eds. Studies in plant science. Vol.4. Advances in plant biotechnology, Elsevier, 1994, pp281–305.

[6] P. Wilson, "The pilot-scale cultivation of transformed roots," in P. Doran, Eds. Hairy roots: Culture and applications. Amsterdam: Hardwood Academy Publishers, 1997, pp. 179–190.

[7] P. Weathers, T. Smith, D. Hemmavanh, E. Follansbee, J. Ryan and R. Cheetham, "Production of the antimalarial, artemisinin, by transformed roots of Artemisia annua," Acta Hort, vol. 426, pp. 157–163, 1996.

[8] M. Jaziri, K. Shimomura, K. Yoshimatsu, M. Fauconnier, M. Marlier and J. Homes, "Establishment of normal and transformed root cultures of Artemisia annua L. for artemisinin production," J Plant Physiol, vol. 145, pp. 175–177, 1995.

[9] C. Z. Liu, Y. C. Wang, X. Z. Kang and F. Ouyang, "Investigation of light, temperature and cultivated modes on growth and artemisinin synthesis of Artemisia annua L shoots," Acta Phytophy. Sin. Vol. 25, pp. 105–109, 1999.

[10] T. C. Smith, P. J. Weathers and R. D. Cheetham, "Effects of gibberellic acid on hairy root cultures of Artemisia annua: growth and artemisinin production," In Vitro Cell. Dev. Biol. Plant, vol. 33, pp. 75–79, 1997.

[11] P. J. Weathers, G. Bunk and M. McCoy, "The effect of phytohormones on growth and artemisinin production in Artemisia annua hairy roots," In Vitro Cell Dev. Biol. Plant, vol. 41, pp. 47-53, 2005.

[12] P. J. Weathers, R. D. Cheetham, E. Follansbee and T. Teoh, "Artemisinin production by transformed roots of Artemisia annua," Biotechnol. Lett, vol. 16, pp. 1281–1286, 1994.

[13] G. Q. Cai, G. Z. Li, H. C. Ye and G. F. Li, "Hairy root culture of Artemisia annua L. by Ri plasmid transformation and biosynthesis of artemisinin," Chin. J. Biotechnol, vol. 11, pp. 315–320, 1995.

[14] O. L. Gamborg, R. A. Miller and K. Ojima "Nutrient requirements of suspension cultures of soybean root cells," Exp. Cell Res, vol. 50, pp. 151-158, 1968

[15] T. Murashige and F. Skoog, "A revised medium for rapid growth and bioassays with tobacco cultures," Physiol. Plant, vol. 15, pp. 473-497, 1962.

[16] J. D. Litvay, D. C. Verma and M. A. Johnson, "Influence of a loblolly pine (Pinus taeda L.) culture medium and its components on growth and somatic embryogenesis of the wild carrot (Daucus carota L.), " Plant Cell Report, vol. 4, pp. 325-328, 1985.

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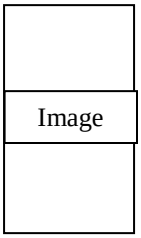

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