

# Establishment hairy root culture system of *Salvia miltiorrhiza*

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## Abstract

*Salvia miltiorrhiza* Bunge (Labiatae) is one of the most important traditional Chinese medicine for treating cardiovascular related diseases. Hairy root is one kind of genetic transformed root tissues that induced by *Agrobacterium rhizogenes*. The highly productive growth characteristic of hairy root along with genetic stability proved that hairy root is a better culture source than cell suspension for production secondary metabolites. Factors affecting growth efficiency of *S. miltiorrhiza* hairy root culture were investigated in this study.

## Materials & Methods

**Plant material**-Hairy root lines induced from *in vitro* *S. miltiorrhiza* leaf explant by *A. rhizogenes* strains R1601 were used in this study. Unless specified root tips with 1 cm in length were cultured into B5<sup>1</sup> basal salt medium containing with 3% sucrose and additional 1% agar for solid medium. All cultures were kept in dark at 25°C and liquid culture shaking at 100 rpm.

## Results

### 1. Effect of medium salt formula on hairy root growth of *Salvia miltiorrhiza*

Five salt formula for hairy root culture were tested. The result showed that Woody plant medium (WPM<sup>2</sup>) had the highest fresh and dry weight followed by B5, Murashige and Skoog (MS<sup>3</sup>) and 1/2MS medium (Tab. 1). Undetectable root growth was found on N6<sup>4</sup> medium (Tab. 1 and Fig. 1)

Table 1. Effect of medium salt formula on hairy root growth of *Salvia miltiorrhiza*

Medium salt formula	Fresh weight (g/50 mL)	Dry weight (g/50 mL)
WPM	3.50 a z	0.34 a
B5	1.18 b	0.10 b
N6	0.06 c	0.01 c
MS	1.02 b	0.10 b
1/2MS	1.07 b	0.11 b

<sup>z</sup> Means followed by the same letter are not significantly different at 5% level by LSD test.



Figure 1. Hairy root of *Salvia miltiorrhiza* grew in various salt formula medium for 6 weeks, medium of (a) WPM, (b) B5, (c) N6, (d) MS and (e) 1/2MS.

**2. Effect of sucrose concentration on hairy root growth of *S. miltiorrhiza***- Root growth was found increased along with sucrose concentrations and the highest fresh and dry weight were both obtained from 6% sucrose treatment after 6 weeks of culturing (Tab. 2 and Fig. 2).

Table 2. Effect of sucrose concentration on hairy root growth of *Salvia miltiorrhiza*.

Concentration of sucrose (%)	Fresh weight (g/50 mL)	Dry weight (g/50 mL)
0.0	0.00 d z	0.00 d
1.5	1.36 c	0.11 d
3.0	3.41 b	0.30 c
4.5	4.44 a b	0.44 b
6.0	4.90 a	0.57 a

<sup>z</sup> : Means followed by the same letter are not significantly different at 5% level by LSD test.

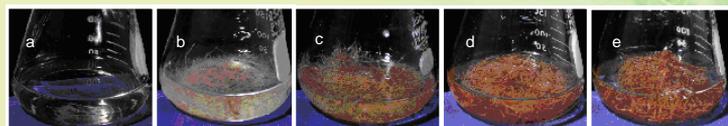


Figure 2. Effect of sucrose concentration on hairy root growth of *Salvia miltiorrhiza*. Hairy root grew on medium containing with (a) 0.0, (b) 1.5, (c) 3.0, (d) 4.5 and (e) 6.0% sucrose for 6 weeks.

**3. Effect of liquid and solid type of medium on hairy root growth of *S. miltiorrhiza***- Hairy root growth in liquid culture was found with 3 times higher on fresh weight and 6 times higher on dry weight than that of in the solid culture (Fig. 3).

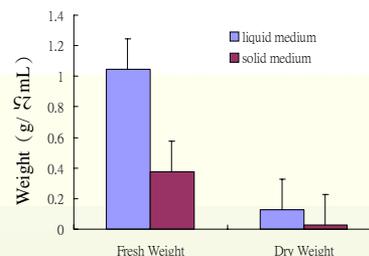


Figure 3. Effect of type of culture medium on hairy root growth of *S. miltiorrhiza*.

### 4. Effect of illumination culture condition on hairy root growth of *S. miltiorrhiza*

Hairy root growth was found with 1.5 times higher on fresh weight in light culture condition than that in darkness (Fig. 4) however organogenesis was found for some of hairy root lines cultured under the light (data not shown).

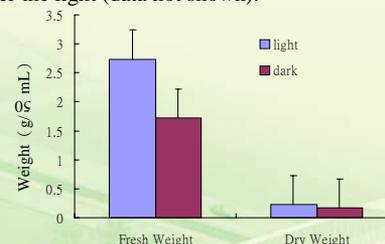


Figure 4. Effect of light and dark culture condition on hairy root growth of *S. miltiorrhiza*.

**5. Time course of *S. miltiorrhiza* hairy root growth**- The growth of hairy root was measured every week up to 12 weeks. All three lines showed continuous growth before 10 weeks on fresh weight and line HR-1 was not ceased growth yet at the end of 12 weeks culture (Fig. 5).

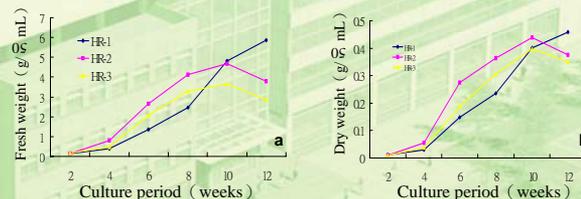


Figure 5. Growth course of *S. miltiorrhiza* hairy root lines HR-1, HR-2 and HR-3, on (a) fresh weight and (b) dry weight.

## Conclusion

The maximum biomass for hairy root growth was achieved at 10 to 12 weeks culture depending to the hairy root lines. In conclusion of factors tested in this study, the ultimate growth of hairy root is to use WPM liquid medium containing 6.0% sucrose under light condition for at least 10 weeks of culture.

## References

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