

Summary

Corydalis yanhusuo W. T. WANG with the common name of Corydalis Rhizoma or Yanhusuo, belonging to *Corydalis* spp. in the family of Fumariaceae (or Papaveraceae), is one of the most important Chinese medicinal herbs. It is a perennial herb but usually cultivated as an annual crop using tubers. The tuber of Yanhusuo containing dl-tetrahydropalmatine (THP) and D-corydaline (COR), a source of pharmacologically important alkaloids, are used as neuroactive alkaloids for treating analgesic and hypnotic actions. It is thought with different climate or geological environment may effect the plant growth and result in its alkaloid production. Therefore, culturing of *C. yanhusuo* by tissue culture to get high yield as well as a constant production of alkaloids has been considered. To achieve these goals, studies of callus induction, suspension cell culture, somatic embryo development, tuber formation, induction of somatic embryos on converted somatic embryos and bioactive alkaloid production have been established and the results were described as below : (1) Callus induction rate as high as 100% was obtained using the tuber cultured on a medium containing basic MS salts, 3% sucrose, 0.9 % agar , 2 mg/L BA, 1 mg/L 2,4-D. (2) Callus starting to grow at 7th-9th day after transplanting was observed and it got into log phase at the 12th day. The most proper incubation period was considered between 26-30 day since the highest weight (8 times) was obtained after 36 day culturing. (3) A medium containing basic MS salts, 5-10 mg/L tyrosine, 3 % sucrose, 0.9 % agar was found the most suitable medium for callus growth as well as for producing secondary metabolites of tetrahydropalmatine and corydaline. (4) Although light treatment inhibited callus growth , it slightly increased the production of corydaline and tetrahydropalmatine. In light treatment, callus appeared slightly red in color with the tendency for forming somatic embryos. (5) The loose and soft callus for cell suspension was obtained on a medium containing

basic MS salts, 3% sucrose, 0.9 % agar and 1 mg/L 2,4-D. (6) The optimum medium for cell suspension culture was contained on a medium containing MS basal salts, 3% sucrose and 1 mg/L 2,4-D with pH at 5.2. Cultures were maintained at 25 ± 1 under $10 \mu\text{E}/\text{M}^2\text{S}$ light intensity with a 14 hr photoperiod for vigorous growth. The shaking speed was suggested to be 80-120 rpm. (7) A medium containing 0.5 mg/L kinetin increased the number of somatic embryos under the light incubation, however the consecutive cultured callus would gradually lost its differentiation ability for inducing somatic embryos. (8) **Somatic embryos on converted somatic embryo** were induced on medium containing 2 mg/L ABA. A 80 % germination rate was observed for somatic embryo when they cultured on a medium containing 0.1 mg/L GA₃ after 2 months. (9) Somatic embryos converted to plantlets along with a good tuber formation on a medium containing 1/2 MS, 6% sucrose and 0.9% agar medium after 1-2 months was observed. Plantlets with tubers was transferred to the medium containing with MS, 3% sucrose and 0.2% gelrite for growing up. A 5% plant with flower bud formation was observed in the flask after 2-4 months culturing. (10) After 4-6 months of culturing, well-developed tubers were transplanted to plastic trays with a potting mixture of peat moss : vermiculite 1:1 (v/v) in green house. A 82% survival rate of *in vitro* plantlets were obtained after 2-month culturing. (11) Supplying amino acid precursor, 5-10 mg/L tyrosine, could further improve production of THP and COR in callus or suspension cultures. (12) In conclusion, by using appropriate growth regulators and prolonged incubation the converted somatic embryo on a medium containing 1/2 MS, 6% sucrose and 0.9% agar was able to produce the good quality tubers with a high and uniform content of THP and COR which could be used for pharmacological studies.