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High frequency plant regeneration from hairy root lines of *Catharanthus roseus* via somatic embryogenesis

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Objectives

To establish the high frequency plant regeneration system from hairy root lines of *Chtharanthus roseus*, hairy root lines (2N-1, 43, 64, and 258) were cultured on MS medium supplemented with several concentration of 2,4-D. Plants were successfully regenerated from hairy root line 64 via somatic embryogenesis.

Materials and Methods

1. Material

Plant - *Catharanthus roseus*- hairy root lines (2N-1, 43, 64, and 258)

2. Methods:

Hairy root segments (about 1 cm) were cultured on MS medium supplemented with 0, 0.1, 0.3, 0.5, 1, 1.5 and 3mg/L of 2,4-D, respectively. Each callus derived from root lines were transferred to MS basal medium. After 4 weeks of culture, the frequency of somatic embryo formation was determined.

Results and Discussion

Root explants from hairy root lines of *Catharanthus roseus* formed white friable calluses after 4 weeks of culture on MS medium supplemented with various concentration of 2,4-D. Calluses derived from hairy root line were transferred to MS basal medium. The highest callus formation occurred onto the MS medium supplemented with 1 mg/L 2,4-D. However, these calluses did not produce somatic embryos upon transfer to MS basal medium. Whereas calluses derived from hairy root lines formed somatic embryos when they cultured on MS medium supplemented with 0.5 mg/L of 2,4-D. Globular to heart-shaped somatic embryos formed on callus were subsequently developed into cotyledonary somatic embryos. Somatic embryos were regenerated into plantlets at a frequency of approximately 90 % and rooted successfully without any treatment. The frequency of somatic embryo formation between hairy root lines showed a great difference. One of them, hairy root line 64, was capable to produce more somatic embryos than other hairy root lines. Therefore, hairy root line 64 will be directly applied to development of transgenic *C. roseus* plant for metabolic engineering of indole alkaloids. It is also remained to examine the genetic and environmental causes underlying the difference of embryogenic potential between hairy root lines.

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