Chapter 38
Hairy Roots

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ABSTRACT

Agrobacterium rhizogenes induces hairy root disease in plants. The neoplastic (cancerous) roots produced by A. rhizogenes infection, when cultured in hormone free medium, show high growth rate and genetic stability. These genetically transformed root cultures can produce levels of secondary metabolites comparable to that of intact plants. Several elicitation methods can be used to further enhance the production and accumulation of secondary metabolites. Thus, hairy root culture offer promise for high production and productivity of valuable secondary metabolites in many plants. Hairy roots can also produce recombinant proteins from transgenic roots, and thereby hold immense potential for pharmaceutical industry. Hairy root cultures can be used to elucidate the intermediates and key enzymes involved in the biosynthesis of secondary metabolites, and for phytoremediation due to their abundant neoplastic root proliferation property. Various applications of hairy root cultures and potential problems associated with them are discussed in this chapter.

INTRODUCTION

Hairy root is a disease of higher plants caused by the bacterium *Agrobacterium rhizogenes*, a gram negative soil born bacterium. When the bacterium infects the plant, the pathogen transfers a DNA segment (T-DNA region bounded by 25 bp direct oligonucleotide repeats) from its large root-inducing (Ri) plasmid into the infected plant. The T-DNA integrates into the nuclear genome of the host plant. The T-DNA carries a set of genes that code for enzymes for the phytohormone auxin control and cytokinin biosynthesis (*iaaM*, *iaaH*, *ipt*) and also for opines (an unusual amino acids). Expression of these new hormones induces the formation of proliferated roots. These roots emerge at the wound sites and are called hairy roots (HR).The mechanism of transfer of T-DNA from *A. rhizogenes* to the host plant is similar to the mechanism involved in *A. tumefaciens,* which causes crown gall disease in the infected plants.

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The hairy roots have unique characteristics like fast hormone independent growths, lack of geotropism, lateral branching, and genetic stability. Further, hairy roots can be cultured under *in vitro* conditions indefinitely. Because of these characteristics hairy roots cultures were investigated for several decades to exploit the possibilities of their commercial use. One of the objectives was to exploit the production of high value secondary metabolites. Long term aseptic hairy root cultures have been established from more than 200 plant species, having the ability to synthesize a wide variety of secondary metabolites and to adjust their metabolic activities in response to biotic and abiotic stress. However, it is important to note that not every hairy root culture displays these characteristics. Further, many researchers have experienced problems with hairy root initiation and maintenance. But the overwhelming positive results have generated hope for its utilization.

In some species, regeneration of whole plants has been possible from the hairy roots. Transgenic plants have been obtained in 89 different plant taxa, representing 79 species from 55 genera and 21 families, through *A. rhizogenes* mediated transformation. The transgenic plants show a characteristic phenotype, called HR syndrome, which include reduced apical dominance in stems and roots, shortened internode, high growth rate of roots in culture, wrinkled leaves with increased width to length ratio, plagiotropic roots, with altered geotropism, altered flower morphology, late flowering, reduced fertility and reduced pollen and seed production.

HAIRY ROOT INDUCTION AND SELECTION

Procedures for induction and selection of hairy roots are described in the following section.

Establishment of Hairy Root Culture System

Establishment of hairy root culture system involves several requirements. These include: the bacterial strain of *Agrobacterium rhizogenes*, an appropriate explant, a proper antibiotic to eliminate redundant bacteria after cocultivation, and a suitable culture medium. On the basis of types of opines produced, *A. rhizogenes* strains can be divided into five lines: octopine, agropine, nopaline, mannopine, and cucumopine. Owing to their strongest hairy root induction ability, agropine strains are the most often used strains. Explants from leaf, stem, shoot tip, stalk, petiole, cotyledon, hypocotyls, tubers, and protoplast can be used to induce hairy roots. However, selection of right type of explants and its age are critical for the success of induction of hairy roots. Juvenile material is the preferred choice.

For induction of hairy roots, explants are separately wounded and cocultivation or inoculated with *A. rhizogenes*. After 2-3 days, the explants are transferred into solid media with antibiotics, such as cefotaxime sodium, carbencilli disodium, vancomycin, ampicilin sodium, claforan, streptomycin sulfate, or tetracycline. Antibiotics are used to kill or eliminate the redundant bacteria at a concentration ranging from 100 to 500 g/ml. Depending on the plant species and type of explants used, hairy roots are normally induce within a week to over a month. Hairy roots thus induced can be sub-cultured on phytohormone-free medium.

To activate the virulence genes of *A. rhizogenes* and to enhance the transfer of foreign genes into the plant genomes, acetosyringone (ranging from 10 to 150 μM) has been used (Kumar et al. 2006).

For high production of secondary metabolites, optimization of the nutrients in the culture medium and physical factors of the culture, are equally important. Factors such as carbon source and its concentra-