Forest Tree Gene Transformation Researches in Japan

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Introduction

Forests are valuable, not just for the wood products they supply now but also as an environmental resources. There are therefore different expectations of the results of gene transformation in forest trees. These include increasing commercial timber values based on sound growth and greater strength, and reducing lignin content and increasing cellulose contents in wood to be used by the pulp industry. From an environmental perspective, an increased ability to fix carbon dioxide is desirable to cope with the problem of global warming. Insect resistance and drought resistance are additional targets in terms of both wood usage and environmental considerations.

Situation in Japan

The application of plant gene transformation is very limited in Japan; for forest trees this approach has been restricted to laboratory studies, and there have been no field trials of genetically modified (GM) trees in Japan. Here we present the current status and objectives of forest tree gene transformation researches.

Current status

How to introduce alien genes

There are two major gene transformation methods for forest trees, as for other plant species. These are *Agrobacterium* transformation, which is a transformation system of bacterium and biolistics, which is the injection of DNA into cells by mixing DNA with small metal particles and firing this combination into the host cell at very high speeds.As there are many tree species, gene transformation research efforts are of necessity focused on a limited number. One of the better-studied species is the poplar. This tree is very suitable for this purpose because culturing its tissue and the transformation of genes using *Agrobacterium* are easy. It was the first GM tree among hardwood species. Although *Agrobacterium* transformation is stable in this tree, most coniferous species are resistant to this bacterium. To overcome this problem we have developed an Agrobacterium transformation system using embryogenic tissue from major Japanese coniferous species. Figure 1 shows *Agrobacterium* transformation of the green fluorescent protein (GFP) gene using embryogenic tissue in the cypress, *Chamaecyparis obtusa*. Somatic embryos were induced and germinated, successfully expressing the GFP gene. The left panel of Figure 1 shows a germinated somatic embryo and the right shows the same embryo exhibiting GFP fluorescence under a mercury lamp.

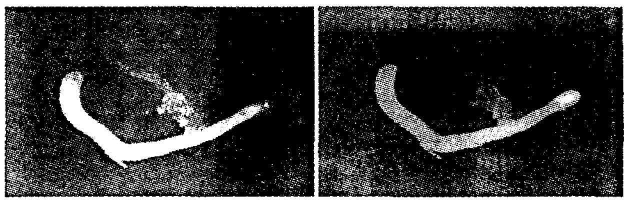

Figure 1. *Agrobacterium* transformation of the GFP gene in *Chamaecyparis obtusa.*

Left: Germinated somatic embryo, Right: GFP fluorescence under a mercury lamp.

In hardwood species, we have already succeeded in *Agrobacterium*-mediated gene transformation in the species *Quercus acutissima*, which is used as a bed-log for shiitake mushroom cultivation. Biolistic transformation has also been applied to many economically important plants. Although this technique is simple and applicable to any kind of organs, only transient expression of reporter genes has been observed in our laboratory.

Embryogenesis of forest trees

As embryogenic tissues are suitable for gene transformation, embryogenesis of coniferous trees has been developed. This first succeeded in the spruce, *Picea abies* (von Arnold *et al.*). Somatic embryos are easily obtained in this genus. Recently, using Smith's medium with some modifications, embryogenesis has been induced successfully in some Japanese coniferous tree species, such as *Cryptomeria japonica, Chamaecyparis obtusa, Pinus thunbergii and Larix kaempferi.* In the case of hardwood species, an embryogenesis system has been developed for *Quercus acutissima* and for *Eleutherococcus sciadophylloides,* a wild vegetable that is also used for wood-carving. Agrobacterium transformation has become easier with the establishment of these embryogenesis systems. As biolistic transformation has also been proven successful using embryogenic tissues of Pinus radiata (Walter *et al.*), an embryogenesis system might contribute to the further development of this approach in other coniferous tree species.

Objectives of forest tree gene transformation research

GM tree research in Japan first focused on reducing lignin content. The lignin content of GM poplars with an introduced antisense peroxidase gene was decreased from 3% to 26% compared with controls (Yahong *et al.*). An introduced xyloglucanase gene was proven to influence cellulose

contents in poplars (Hayashi *et al.*). pollen of coniferous tree species can disperse widely, a pollen-free GM tree is desirable to prevent crossing between GM and normal trees.

Increasing insect resistance remains important. As there is much damage caused by moth larvae, introducing the *Bacillus thuringiensis* toxin gene is one desirable goal for the genetic modification of *Chamaecyparis obtusa.* At least 10% of Japanese people are allergic to the pollen of *Cryptomeria japonica* and *Chamaecyparis obtusa.* To cope with this problem we aim, by using gene transformation, to prevent male flower formation. Moreover, as the

Current circumstances of research

The Japanese government ratified its 1992 signing of the Cartagena Protocol on Biosafety to the Convention on Biological Diversity in 2003. The related law and rule to this protocol came into effect on 19 February 2004. Before this, there were only guidelines for transformation research; all researchers should now observe the protocols. Public acceptance is another issue of transformation research. Under such circumstances, the onus is on scientists to explain our research, both to retain present levels of research activity and to contribute to human happiness and prosperity in the future.