Engineering Poplar Plants for Phytoremediation

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Abstract: Poplar plants are naturally resistant to heavy metals and other contaminants of environment. They grow fast and produce large biomass. Moreover, they can be engineered to express foreign genes, and thereby modified to exhibit characteristics desirable for remediation of environment. We expressed many heavy metal tolerance genes in a line of poplar that does not flower, and are in the process of testing them in greenhouse and in the field.

Keywords: Poplar, Phytoremediation of heavy metals, Genetic engineering

1. Introduction

Our environment is contaminated by diverse pollutants including industrial by-products such as heavy metals, waste matters, fertilizers and pesticides. The problem of pollution is not likely to disappear soon since the world's population continues to grow and their need for improved living standard also continues to increase, which causes further pollution. Therefore, it is necessary to invent improved method to remedy polluted environment. The conventional physical or chemical cleanup of the widespread contaminated area is costly and can often further damage the environment. Phytoremediation was proposed as a low-cost, environmentally-friendly way to remove pollutants from contaminated soils [1]. The use of plants costs less than the use of any other forms of organisms, since plants capture solar energy to synthesize the proteins and other structures necessary for the remediation. Moreover, plants are ascetically pleasing, and have good influence on the physical, chemical and biological aspects of the environment.

In the USA and Europe, plants that naturally hyper-accumulate toxic materials [2, 3] are used for the purpose of remediation of the polluted environment. They include poplar, Indian mustard, and willow trees. However, phytoremediation using these natural plants has limitations. The plants may suffer toxicity symptoms, and it takes long time for the plants to grow to certain size to effectively extract the pollutants. Therefore, it would be beneficial to enhance the plant's capacity for phytoremediation. Genetic engineering provides an excellent means to achieve this goal. It is possible to generate transgenic plants that can express exogenous genes or overexpress its own genes and thereby acquire new or improved phenotypes. Many transgenic plants with new characteristics due to expression of genes from microorganisms, yeasts, mouse, or even human have been reported in scientific journals [4-6]. Once an individual plant with suitable phenotypes is produced, it is easy to multiply the numbers of plants by cloning methods, such as cuttings and somatic embryogenesis. Moreover, many trees continue to grow for many years as long as the conditions are suitable.

We already found many genes that enhance tolerance to cadmium, lead, or zinc, when overexpressed in Arabidopsis [7-14]. We introduced many of the genes into poplar, a fast growing, large biomass plant, and are now testing their potential as a remediation agent.

2. Results and Discussion

1) Plant Materials and Transformation

Poplar was used because the species grows fast into large biomass and is easy to propagate by cutting. To minimize the concerns on spreading of transgenic trees to the environment, we used 'Bonghwa' poplar, a natural non-flowering mutant of hybrid poplar (*Populus alba* X *P. tremula var. glandulosa*), which was identified by Korea Forest Research Institute (KFRI) (Fig.1).

Non-flowering mutant poplar (Bonghwa)

Normal flowering poplar of the same age

Fig. 1 Bonghwa poplar (left) does not develop flower, while normal poplar at the same age flowered and produced seeds.

Poplar plant transformation was conducted using *Agrobacterium*-mediated transfer of genes. In brief, young aseptically-grown poplar plants were excised to obtain internodes, which were then co-incubated with *Agrobacterium* which harbors the metal-resistance gene and the antibiotics resistance gene for selection. For the first two days of incubation, light was blocked to increase the efficiency of transformation. After calli develop at the cut ends of the internode, they were transferred to shoot induction medium [2.46 g/L WPM (Woody Plant Medium), 1 mg/L Zeatin, 0.1 mg/L BAP, 0.01 mg/L NAA, 3% sucrose, pH 5.5] with appropriate antibiotics to regenerate shoot, and then to root induction medium (2.2 g/L MS, 0.2 mg/L IBA, 3% sucrose, pH 5.8) with appropriate antibiotics to induce root development [15]. Transformed poplar seedlings were genotyped for the presence of transgenes and the expression of introduced genes was checked by using RT-PCR. Transgenic poplar plantlets which express genes of interest were then multiplied by cuttings. We have successfully generated transgenic poplar trees which express YCF1, a yeast ABC transporter which transports toxic metals into the vacuole, and many more genes.

2) Test of heavy metal resistance of young plantlets in test tube

The performance of transgenic poplar plants in phytoremediation was first tested in test tube trials. The young plantlets of WT and transgenic poplar at similar sizes were chosen for comparison. YCF1 is an ABC transporter found in baker's yeast (*Saccharomyces cerevisiae*) and involved in transport of toxic materials like heavy metals into the vacuole. We have demonstrated that YCF1-expression improves the resistance to Cd and Pb in *Arabidopsis thaliana* by increasing vacuolar sequestration of those metals [9]. The expression of YCF1 also resulted in the enhancement of Cd-tolerance in poplar, similarly as in Arabidopsis plants. When grown in agar media containing 0.1 and 0.3 mM of $CdCl_2$, the poplar plants which express YCF1 grew much better than the wild type plants did (Fig. 2). This suggests that heavy metal tolerance genes which were selected using *Arabidopsis* system will work well in tree system, especially in poplar.

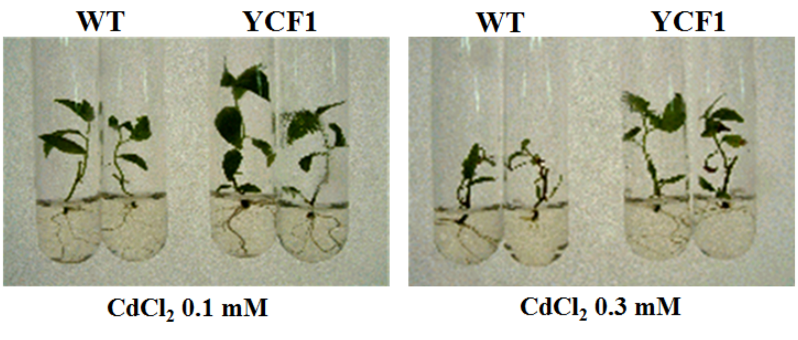

Fig. 2 Test of heavy metal tolerance of transgenic poplar in test tubes. Wild-type (left) and YCF1 (right) poplar seedlings were grown in $CdCl_2$ 0.1 and 0.3 mM containing media. Expression of YCF1 dramatically increased Cd-tolerance in poplar.

3) Test of heavy metal resistance of soil-grown plants in a greenhouse

Poplar seedlings which were grown in the sterilized condition of test tubes can be acclimated to soil in a green house. With soil-grown poplar plants, we can test their capacity of heavy metal tolerance for a longer period with a larger biomass. WT and transgenic poplar plants with similar growth were chosen and soaked in solutions containing heavy metals on a regular basis. Consistent with test results in test tubes, YCF1 poplar plants grew better than WT and accumulated more Cd (data not shown).

4) Field Test of heavy metal resistance of transgenic poplar plants

We selected transgenic poplar lines with enhanced heavy metal tolerance and accumulation based on the experiments in the controlled environment and planted them in our test site for multiple-year field trial. Our test site is located in a remote closed mine area, which is contaminated by heavy metals such as arsenic, cadmium, zinc and other toxic elements. To protect the transgenic poplars from wild life and also to prevent escape of the fallen leaves or branches from the test site, we erected a barbed-wire fence with a dense net around the test site (Fig. 3).

Fig. 3 The fenced field test site in a closed mine area.

In the first year of planting, survival rates of wild-type and transgenic poplar were counted. High survival rate is important for the application of transgenic trees to remediate heavily contaminated soils. After one year of adaptation, growth of the poplar trees in test site was monitored regularly (Fig. 4). The height of individual transgenic and wild-type poplar trees was measured. The capacity to accumulate heavy metals was also tested by measuring heavy metal contents in leaves. At the same time, heavy metal contents of the soil were also measured to check whether poplar trees can decrease the concentration of heavy metal in the surrounding soil.

Fig. 4 Transgenic and wild-type poplar trees were planted in the test site. The growth and heavy metal accumulation of plants is monitored regularly. Black plastic sheets cover the soil surface to maintain high humidity for efficient growth of poplar trees and to inhibit the growth of weeds.

3. Conclusions

After multiple-year field trials, we will understand whether our transgenic poplar plants are indeed improved in the capacity to remediate the polluted environment, and thus suitable for actual phytoremediation. There is a possibility that our plants with single gene insertion may not be sufficiently improved in the capacity to remediate the actual field, although they were very effective in our greenhouse trials. The strongly-effective phytoremediation may be possible only when multiple genes with different and synergistic mechanisms of phytoremediation are expressed at the same time. To develop more and better plants for phytoremediation, we continue to search for new genes with different functions and stack them (introducing many genes into the same line of plants), which will hopefully further improve the plants' capacity to clean up the environment.

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