A Wide Cross-Tolerance of Transgenic Rice Containing Human CYP2B6 to Various Classes of Herbicides

Sakiko Hirose and Hiroyuki Kawahigashi*

Pesticides have played very important roles against food shortages and plant diseases, and our modern life would be vastly different without their use. Weed infestation adversely affects crop production by reducing yields and decreasing market prices of the crop. For cost-effective land use, crop yield and price must be maximized, and the cost of weed control minimized. Herbicides substantially lighten the farmer’s heavy physical workload and improve crop yield and product quality. Herbicides are now widely used for crop cultivation and for management of lawns, railroad rights-of-way, highway margins, and other purposes, although there remains the possibility of non-target effects of pesticides to the environment caused by agricultural runoff.

One of the major problems with the use of herbicides is that repeated use of the same herbicide in a field tends to promote the emergence of herbicide tolerant weeds. Indeed, over 290 biotypes of herbicide tolerant weeds have been reported in agricultural fields and gardens worldwide (International survey of herbicide tolerant weeds, http://www.weedscience.org/in.asp). They are categorized into 18 HRAC (The Herbicide Resistance Action Committee) groups (Classification of Herbicides According to Mode of Action 2005, http://www.plantprotection.org/hrac/Bindex.cfm?doc=moa2002.htm). Fifty percent of the biotypes are resistant to inhibitors of acetolactate synthase, such as sulfonylureas, and inhibitors of photosynthesis at photosystem II, such as triazines.

Several strategies are used to prevent or reduce herbicide tolerance in weed populations. Using either several herbicides in rotation or in a mixture including lower doses of herbicides was proposed as the most practical approach to prevent or delay the appearance of tolerance, because most cases of herbicide tolerance are due to a single major gene, and also because the repeated use of a single herbicide for a long time tends to promote weeds that are tolerant.

To enable crop plants to grow under such an herbicide regime, it would be helpful to develop transformed plants containing a gene for a single mammalian P450 enzyme, which would detoxify several types of herbicides, and thereby give these plants cross-tolerance to herbicides.

The cytochrome P450 monooxygenase (P450) system, consisting of cytochrome P450 and NADPH-cytochrome P450 oxidoreductase, catalyzes monooxygenation of lipophilic xenobiotic compounds, including herbicides. It has been reported that several microsomal P450s are involved in xenobiotic metabolism in mammals. The individual xenobiotic-degrading P450s appear to show overlapping, broad substrate specificity and thus improve the animal’s ability to catabolize a variety of unknown lipophilic compounds, including herbicides, in liver.

We introduced a human gene for CYP2B6, a cytochrome P450 monooxygenase that inactivates xenobiotic chemicals, into Oryza sativa cv. ‘Nipponbare’ by Agrobacterium-mediated transformation. In our study, we demonstrated that transgenic rice expressing human CYP2B6 under the control of CaMV 35S showed strong herbicide tolerance that resulted from the detoxification of several types of herbicides by the CYP2B6.

The CYP2B6 rice plants showed normal growth in morphology, including plant height, leaf color, flowering time, fertility, and seed size, compared with non-transgenic Nipponbare plants in a greenhouse. The CYP2B6 rice plants were physiologically the same as non-transgenic Nipponbare, except for the feature derived from the introduced CYP2B6 gene.

The CYP2B6 rice plants showed high tolerance to 13 out of 17 herbicides, which belong to different chemical families. These were chloroacetoamides (acetochlor, alachlor, metolachlor, pretilachlor, and thentylchlor), oxyacetamides (mefenacet), pyridazinones (norflurazon), 2,6-dinitroanilines (trifluralin and pendimethalin), phosphoamidates (amiprofos-methyl), thiocarbamates (pyributicarb), and ureas (chlortoluron).

Significantly high tolerance was observed to the five chloroacetoamides, which inhibit the synthesis of very long chain
fatty acids (VLCFA). For example, the CYP2B6 rice seeds were able to germinate and grow in the medium containing 80 μM metolachlor (about 15 times the dose of practical use in cornfields), while non-transgenic Nipponbare did not germinate in the presence of 2 μM metolachlor (about one-third the dose of practical use in cornfields). Another VLCFA synthesis-inhibiting herbicide, mefenacet, inhibited the germination of Nipponbare, but had little effect on the growth of CYP2B6 rice plants.

Microtubule assembly-inhibiting herbicides, pendimethalin and trifluralin, and unknown function herbicide, pyributicarb, inhibited the root growth of Nipponbare, but CYP2B6 rice plants produced roots and grew better than Nipponbare. A photosynthesis-inhibiting herbicide, chlortoluron, inhibited the growth of Nipponbare plants, but the CYP2B6 plants grew vigorously.

CYP2B6 rice was slightly tolerant to norflurazon, which caused bleaching of shoots of Nipponbare by the inhibition of carotenoid synthesis. The CYP2B6 rice metabolized the herbicides during and after germination, keeping the concentration of the herbicide in plant tissues under the lethal threshold. Therefore, the CYP2B6 rice plants could metabolize a broad spectrum of herbicides and showed cross-tolerance to several herbicides having different chemical structures and different modes of action.

The results of thin layer chromatography analysis revealed that the amounts of metolachlor decreased in CYP2B6 rice plants and in the medium of CYP2B6 rice faster than those of non-transgenic Nipponbare. In this study, the CYP2B6 rice plants rapidly metabolized metolachlor to its demethylated metabolite. The metabolism of metolachlor seemed to be enhanced by the introduced CYP2B6 in the transgenic plants, although metolachlor was metabolized not only by CYP2B6 rice but also by control plants.

In the greenhouse, CYP2B6 rice plants grew vigorously in an enamel pot with soil and water that also contained metolachlor at the same dose of practical use in cornfields. On the other hand, non-transgenic Nipponbare plants were almost killed by metolachlor. This result indicated that CYP2B6 rice plants were practically useful as an herbicide tolerant crop under the conditions of a paddy field.

The wider cross-tolerance to herbicides having different modes of action and different chemical structures seems to be a special feature of transgenic plants expressing mammalian P450 genes. This cross-tolerance would prove useful to prevent the development of herbicide resistance of weeds, because the use of several herbicides in rotation would not harm the crop.

The herbicide cross-tolerance during germination should be important for weed control in rice fields, especially with the direct-seeding system. In the transplanting–cultivation system of rice seedling, standing water in paddies prevents the germination of many weeds, and as a result, few kinds of plants are major weeds. However, in the direct-seeding system without water cover, the germinating rice must compete with many kinds of weeds. The transgenic rice with cross-tolerance to various types of herbicide should be an ideal plant for weed control with herbicide mixtures, especially in direct seeding.

We expect that CYP2B6 rice will also prove useful in degrading and thus decreasing the environmental loads of herbicides, insecticides, industrial chemicals, and endocrine-disrupting pollutants in paddy fields and the connected water streams. In the future, transgenic plants expressing P450 species should be good not only for developing herbicide-tolerant rice but also for reducing the environmental impact of agrochemicals.

References


Sakiko Hirose and Hiroyuki Kawahigashi*
Plant Biotechnology Department
National Institute of Agrobiological Sciences
Ibaraki, Japan
*shiwak@affrc.go.jp