EXPRESSION OF ARYLACYLAMIDASE IN TRANSGENIC PLANT

Kiyoshi Hagiwara
Department of Molecular Genetics, National Institute of Agrobiological Resources,
Kannondai, Tsukuba, Ibaraki, 305-8602 Japan

Arylacylamidase (EC 3.5.1.13; aryl acylamide amidohydrolyase) hydrolyzes the amide bond of acylanilide. The enzyme is known to distribute in such divergent groups as microorganisms, insects, mammals and plants, when the enzyme activities are detected using acylanilide derivatives as the substrates.

In higher plants, especially, there has been increasing interest in arylacylamidase because the enzyme has an activity to hydrolyze and detoxify propanil (3,4-dichloropropionanilide; DCPA), which is a selective photosynthesis-inhibiting herbicide used in rice field to control weeds. Rice plants are specifically resistant to propanil as the level of arylacylamidase in the leaves is high. In addition to rice, the enzyme activities, which hydrolyze propanil, have been detected in a few kinds of plants, parsley, tulip, dandelion and bamboo. In these plants, arylacylamidase was purified from parsley and tulip. Purified enzymes were labeled selectively with diisopropylfluorophosphate (DFP), this results indicated the existence of active serine in protein residue.

Using the antiserum against purified enzyme, c-DNA clones were obtained and sequenced from parsley and tulip. The sequences of these two genes have slight homology except in some regions, which is supposed to be active site. This active site is widely conserved in serine proteins. The genes of arylacylamidase were constructed into plant expression vector; PBI121, which was used to transform tobacco plants by an Agrobacterium-mediated method.

Transformed tobacco plants were homogenized and assayed for enzymatic activities. The arylacylamidase activity of transgenic tobacco, which was transformed by tulip gene, was not possible to detect. On the other hand, parsley gene was high. The specific activity of purified enzyme of parsley is higher than that of tulip, with this difference in transgenic plants presumably being the difference of the amount of the expression. Expression of arylacylamidase protein in transgenic plants was also confirmed by imuno-blotting. Herbicide-resistant ability of transgenic plant is now in progress.