Genetic improvement of plants with all plant-derived DNA sequences and its application to polyphenol enrichment

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Lifestyle-related illness could be aborted or relieved by oral intake of nutraceuticals and pharmaceuticals, which are mainly derived from plants. To enrich plants with those substances, genetic engineering is the most promising to achieve with a minimal time, whereas genetically modified (GM) crops are not favorably accepted by consumers especially in Japan. Selectable-marker genes beside objective genes to improve plant ability, are always required for plant transformation. A major concern is the employment of antibiotic-resistant genes as selectable markers, which cannot be denied to be a cause for the generation of antibiotic-resistant bacteria in intestines of cattle. Like and dislike behavior of consumers eventually influences the acceptance of GM crops harboring genes such as for antibiotic-resistance from inedible microorganisms. The combination of selectable markers derived from plants and herbicides harmless to the environment and humankind, are worth to be investigated to shorten periods to create GM crops instead of the technology of selectable-marker excision.

We have paid attention to acetolactate synthase (ALS) [acetohydroxyacid synthase (AHAS)] and herbicides which inhibit ALS enzymatic activity. We have succeeded in transformation of nuclear or chloroplast genome of plants with mutated ALS gene (*At-mALS*) from *Arabidopsis thaliana*, a model plant for research. To strongly express objective genes in nuclei, we have further employed a promoter sequence of gene for actin 2 (*AtACT2*) of *Arabidopsis*. This plant-originated transformation system has been applied to accumulation of polyphenols by enhanced expression of gene for *Arabidopsis* chalcone synthase (*AtCHS*) in *Arabidopsis*, where transcript levels of *AtCHS* have increased approx. 20 times higher in rosette leaves. The content of kaempferol glycosides has increased around 3 times in *Arabidopsis* transgenic with *AtCHS*. This strategy has also been applied to expression of genes for benzalacetone synthase (*RpBAS*) from *Rheum palmatum* and for pentaketide chromone synthase (*IpPCS*), a type-III polyketide synthase from *Ipomoea purpurea*. Enrichment with polyphnols has further tried by exposure of non-transgenic Cruciferae plants to light-emitting diode (LED) at 470 nm and resulted in elevation of expression of genes for enzymes for flavonoid and lignan biosynthesis and of the content of kaempferol glycosides and anthocyanins.

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