

# Targeted mutation breeding of flower color by taking advantage of ion-beam irradiation and genomic information

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Mutation breeding has been used to obtain new plants with characteristics that could not be obtained from original varieties, cross-breeding or gene recombination. Much attention has been paid to the randomness of mutation, and therefore, mutation breeding is often called 'not scientific'. Ion beams, which deposit high levels of energy on a local target, induce a limited number of large and irreparable DNA lesions, resulting in null mutations. Thus, only one trait is expected to be changed from the original phenotype by ion beam treatment. In 2007, we launched a project to overcome the randomness of mutation and to develop logical and efficient mutation breeding by taking advantage of both ion-beam irradiation and genomic information. This research was supported by a five-year grant from the Research and Development Program for New Bio-industry Initiatives of the Bio-oriented Technology Research Advancement Institution (BRAIN).

To achieve our purpose, we focused on flower-color mutations, because flavonoid biosynthesis is one of the most well-known molecular mechanisms in higher plants. First, we envisioned four ideal target plants for mutation breeding. These were glittering carnation, red creeping petunia, and blue-purple and crimson fragrant cyclamen (Figure 1). We then investigated which original varieties to choose as the candidate plants to be mutated with ion beams. We selected those candidate plants by integrating knowledge of genes and their products related to flavonoid biosynthesis and modification. For example, one goal is to create a cyclamen with blue flowers. The genus cyclamen has no delphinidin pigments, which are thought to impart blue color, but some cyclamens have malvidins, which are biosynthesized by methylation from delphinidins. Obviously, cyclamens with malvidins should be selected

as candidate varieties, genes responsible for anthocyanin methylation should be targeted by ion beams, and a mutant possessing delphinidins should be selected. If the selected mutant does not have blue flowers, the mutant could be irradiated once again with ion beams to change another gene related to the pigmentation pathway.

Our project involved five research groups, whose work is reviewed in the following articles. In one set of representative results, a logical and efficient mutation method was established for specifically and efficiently inducing flower color mutants by ion beam irradiation using sucrose pretreatment for anthocyanin accumulation. Several new genes related to anthocyanin biosynthesis and modification, such as acyl-glucose dependent anthocyanin 5-glucosyltransferase (AA5GT), anthocyanin methyltransferase (AMT), and glutathione S-transferase (GST), have been isolated for the first time in carnation or cyclamen (Figure 2, in red). New pigments such as cyanidin 3-glucoside (Cy3G), cyanidin 3-malylglucoside (Cy3MG), delphinidin 3,5-diglucoside (Dp3,5dG), pelargonidin 3,5-cyclicmalyl-diglucoside (Pg3,5cMdG), have been identified as major flower pigments in carnation or cyclamen for the first time. Glittering carnation, red semi-creeping petunia, and new flower colors of fragrant cyclamen were created through this project.

Several new factors that strongly affect flower color were identified. 1) Non-acylation was found in a glittering carnation petal. 2) A loss-of-function mutation in GST caused not only a decrease of pigmentation but also a change in flower color in carnation. 3) Carnation flower color was strongly affected by the function of AA5GT. 4) Delphinidins caused cyclamen flowers to turn red-purple. 5) A decrease in flavonol metabolism caused increased amounts of pigmentation in cyclamen

Abbreviations: AA5GT, acyl-glucose dependent anthocyanin 5-glucosyltransferase; AMT, anthocyanin methyltransferase; Cy3G, cyanidin 3-glucoside; Cy3MG, cyanidin 3-malylglucoside; Dp3,5dG, delphinidin 3,5-diglucoside; GST, glutathione S-transferase; Pg3,5cMdG, pelargonidin 3,5-cyclicmalyl-diglucoside.

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flowers. The articles that follow present detailed results and discussion of these new findings.

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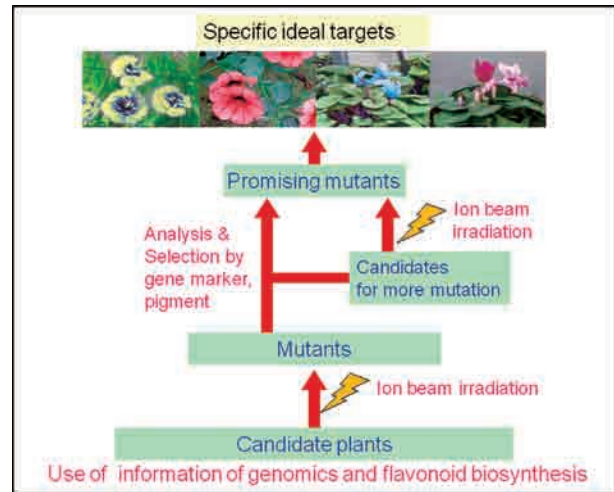


Figure 1. Strategy for targeted mutation breeding.

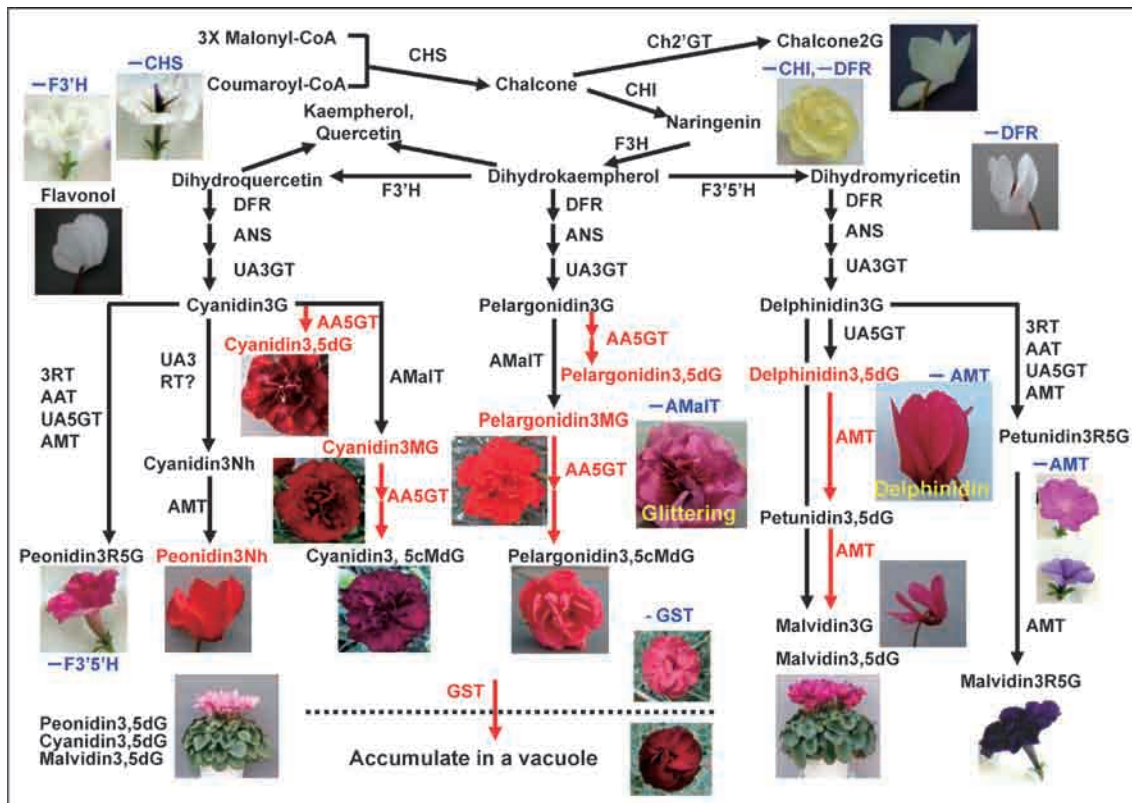


Figure 2. Map of genes and enzymes involved in anthocyanin biosynthesis and modification in some flowers. New genes and enzymes identified in this study are indicated in red. Genes deficient in mutant flowers are in blue. Abbreviations are as follows, CHS: chalcone synthase, Ch2'GT: chalcone 2'-O-glucosyltransferase, CHI: chalcone isomerase, F3H: flavanone 3-hydroxylase, F3'H: flavonoid 3'-hydroxylase, F3'5'H: flavonoid 3',5'-hydroxylase, DFR: dihydroflavonol 4-reductase, ANS: anthocyanidin synthase, UA3GT: UDP-glucose:anthocyanidin 3-glucosyltransferase, AMaIT: anthocyanin malyltransferase, 3RT: anthocyanidin-3-glucoside rhamnosyltransferase, AAT: anthocyanin acyltransferase, UA5GT: UDP-glucose:anthocyanin 5-glucosyltransferase, AA5GT: acyl-glucose dependent anthocyanin 5-glucosyltransferase, AMT: anthocyanin methyltransferase, GST: glutathione S-transferase.