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Developement of various colored fluorescent proteins expressing high intensity in plants

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Objective

This work was done to develop plant expression vectors with high levels of fluorescent color.

Materials and Methods

1. Materials
   Plant - *Arabidopsis thaliana* and *Nicotiana tabacum*
   Agrobacterium tumafacience strain - LBA4404 and C58C1
2. Methods
   Protoplast were obtained from the leaf and root of *Arabidopsis.*
   Mutated sGFP obtained by PCR based point mutation.

Results and Discussion

We have tried to get a mutated sGFP, which expressed red, yellow and blue fluorescence, respectively, and to give transformation to *Arabidopsis.* In order to develop a high-level fluorescent expression system by modification of amino acid, we've made fluorescence with high level more than 100 times and expressed GFP proteins more than 20 times compared to the modified GFP4 (mGFP4). Yellow fluorescent protein (YFP) emitted 529 nm yellow fluorescence in the *Arabidopsis* leaf protoplast developed by nucleotide substitution of S (serine) by G (glycine) in 66th amino acid. Cyan fluorescent protein (CFP) displaced phenol or phenolate of indole, emitted cyan fluorescent. The cyan fluorescence developed by nucleotide substitution of M (methionine) by T (threonine) in 153th amino acid. And blue fluorescent protein (BFP) developed by nucleotide substitution of F (phenylalanine) by L (leucine) in 64th amino acid. Using confocal laser scanning microscopy (CLSM), we investigated the expression of the mutated sGFP, the which had detectable fluorescence in leaf and root protoplasts, showing much higher level than wild type of sGFP by base substitution. Utilizing the *Agrobacterium*-mediated method, transgenic *Arabidopsis thaliana* and *Nicotiana tabacum* expressing visible fluorescent were obtained.

This has demonstrated that various color fluorescent proteins could be developed by the nucleotide substitution in plants.

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