

Metabolic engineering by site-directed mutagenesis via gene targeting in plants

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Using gene targeting (GT), we successfully introduced mutations that improve enzymatic properties into an endogenous rice gene encoding a key enzyme of tryptophan biosynthesis. In mature seeds of GT plants, free tryptophan accumulated to levels up to 230-fold higher than in the original cultivar. This is the first demonstration of the application of GT-mediated targeted mutagenesis to metabolic engineering in crops.

Keywords: metabolic engineering, gene targeting, mutagenesis, tryptophan, rice

Background

Structure-based protein engineering of metabolic enzymes can reveal point mutations that relieve feedback inhibition and enhance enzymatic activity. This information enables the design of modified enzymes with improved enzymatic properties. To date, however, it has not been possible to mutagenize endogenous enzymes as expected in plants. In this study, we demonstrate site-directed mutagenesis of an endogenous plant gene encoding a metabolic enzyme, thus showing that it is indeed possible to perform metabolic engineering in plants. In our gene targeting (GT) test system, we examined whether tryptophan-fortified rice can be produced by site-directed mutagenesis of the *OASA2* gene that encodes a key enzyme in tryptophan biosynthesis of rice.

Results and Discussion

1. A GT vector containing a modified *OASA2* gene encoding a key enzyme of tryptophan biosynthesis (Fig. 1) was transformed into rice (cv. Nipponbare). Cells in which GT occurred successfully were selected with a tryptophan analogue. Thus, we succeeded in the production of novel rice plants in which mutations that relieve feedback inhibition and enhance enzymatic activity were introduced simultaneously into the endogenous *OASA2* gene.
2. In mature seeds of GT plants, free tryptophan accumulated to levels up to 230-fold higher than in the original cultivar (Fig. 2). Moreover, free amino acids other than tryptophan accumulated to levels up to 2- to 10-fold higher than in the original cultivar, suggesting that the whole metabolic pathway of amino acids is modified in GT plants (Fig. 3).
3. Furthermore, in young leaves of GT plants, free tryptophan accumulated to levels up to 7-fold higher than in the original cultivar (Fig. 2), suggesting the possibility that additional tryptophan synthesized and accumulated in the leaves also translocates to seeds in GT plants. This translocation system may be a useful tool with which to accumulate specific metabolites to high levels in seeds.

Future prospects

1. The GT plants produced in this study could be used as a breeding line to produce tryptophan-fortified rice cultivars.
2. Metabolic enzymes involved in amino acid biosynthesis are conserved in various organisms and much basic information on the design of native enzymes has been accumulated. Thus, besides tryptophan, crops fortified with other amino acids could be produced by modification of enzymes involved in amino acid biosynthesis as designed via GT and with subsequent selection on amino acid analogues.
3. The site-directed mutagenesis of endogenous targeted genes based on information from comparative genomics and protein engineering will be a powerful tool in the field of functional genomics and molecular breeding in plants.

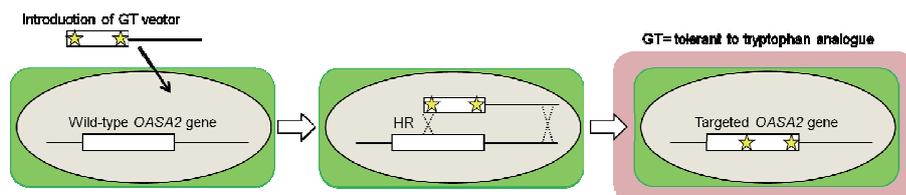


Fig. 1. Modification of OASA2 gene via GT

GT vectors: a 7.0-kb fragment containing a partial OASA2 fragment with mutations (stars) that confer tryptophan insensitivity and enhance enzymatic activity was constructed. This truncated OASA2 was expected to be non-functional, thus recovery of cells showing tolerance to a tryptophan analogue is expected only after homologous recombination (HR) between the GT vector and the chromosomal.

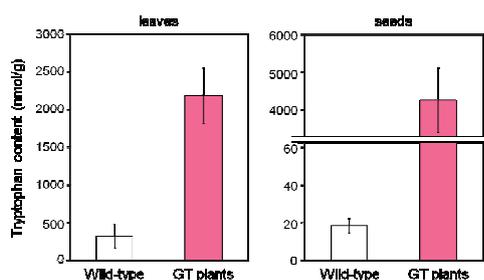


Fig. 2. Free tryptophan contents in leaves and seeds of GT plants

The free tryptophan content in leaves and mature seeds of GT plants is higher than in the original cultivar.

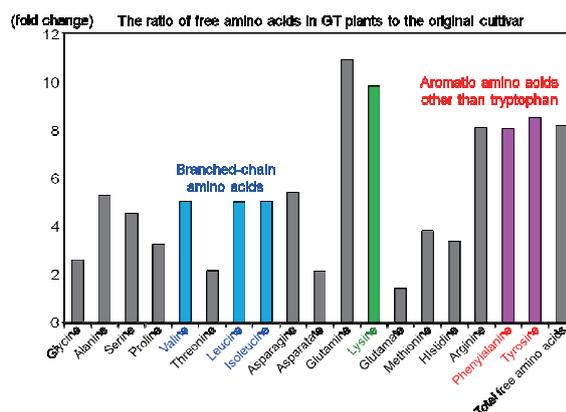


Fig. 3. The ratio of free amino acids in seeds of GT plants to that in the original cultivar

The contents of free amino acids other than tryptophan in mature seeds of GT plants are higher compared to the original cultivar. Overall, the total amount of free amino acids is >8-fold higher than in the original cultivar.

References

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