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Molecular characterization of several genes involving in vitamin A and C biosynthesis in kiwifruit

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Objectives

In order to obtain functional gene materials, we try to isolate several genes involving in vitamin A and C biosynthesis from kiwifruit.

Materials and Methods

1. Material

Plants - Fruits and leaves of kiwifruit (cultivar Hayward)

2. Methods

Probe preparation - RNA extraction, degenerate oligomer design & synthesis, RT-PCR

Gene isolation - cDNA library screening, RACE

Results and Discussion

In order to characterize several genes involving in vitamin A and C biosynthesis in molecular level, we want to isolate carotenoid biosynthetic genes of phytoene synthase and lycopene cyclase, ascorbate biosynthetic genes of GDP-mannose pyrophosphorylase and alactose dehydrogenase. Firstly we obtained gene fragments of respective genes by RT-PCR using degenerate oligomer, designed by multi-sequence alignment.

The sequence was determined and analyzed using bioinformatics. Using the cDNA fragments, we isolated cDNA encoding DGP-mannose pyrophosphorylase and galactose dehydrogenase by cDNA library screening and RACE. The sequence indicated that the one cDNA encoding galactose dehydrogenase was full-length and the other encoding GDP-mannose pyrophosphorylase was partial lacking N-terminal region. The deduced amino acid sequence showed higher level of similarity with potato, tobacco, and citrus. Presently we are introducing these genes into kiwifruit plants.

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