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Development of SYBR green-based real-time PCR markers for verifying *Silybum marianum* and *Cirsium japonicum.*

Joon An[1], Cheol Seong Jang[1*]

[1]Plnat Genomics Lasb, Department of Applied Plant Sciences, Kangwon National University, Chuncheon 24341, Republic of KOREA

[Introduction]

Silybum marianum has other common names, for example milk thistle. This species is an annual or biennial plant of the Asteraceae family. This fairly typical thistle has red flowers and shiny pale green leaves with white veins. Originally a native um of Southern Europe, it is located throughout the world. Milk thistle has been used for a number of purposes including treatment of liver disease. However, in Korea, *Cirsium japonicum* was labeled as milk thistle and sold as food. *Cirsium japonicum* and milk thistle are very difficult to distinguish because they are very similar in morphology after drying. Therefore we tried to classification between *Cirsium japonicum* and milk thistle. Here, we have been developed a species-specific primer sets using the chloroplast genome to classify two plants.

[Materials and Methods]

The seeds of *Cirsium japonicum* were supported by the National Institute of Biological Resources, and the seeds of milk thistle were supported by the institution and we germinated two seeds. Total genomic DNA were extracted from leaves using the i-genomic Plant DNA Extraction Mini Kit (iNtRON Biotechnology, Seongnam, Korea) according to the manufacturer`s protocol. The quantity of the extracts was measured using a SPECTROstar Nano (BMG Labtech, Ortenberg, Germany). Chloroplast DNA sequences were downloaded from the National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/) in order to design primer pairs to amplify these regions of plant. Chloroplast DNA sequences were aligned using ClustalW2 (ftp://ebi.ac.uk/pub/software/clustalw2/). Real-time PCR was performed in a final volume of 20 ul using a QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific). For statistics analysis, implement the standard sample DNA of each species was 10-fold diluted into five series as 0.001-10 ng/ul applied to Real-Time PCR.

[Results and Discussions]

We found SNPs between *Cirsium japonicum* and milk thistle through alignment of chloroplast genes such as r*bcL, ndhA, ycf1*, and *ndhF*. So we produced three pairs of species-specific primers of two plants using SNPs. The sensitivity of primer sets are assessed serially ten-fold diluted of total DNA (including chloroplast DNA) and efficiency analyzed in each primer sets using the regression test. A linear correlation ($R2 > 0.99$) were obtained between the crossing point values and log DNA concentration. We applied the developed method to the commercial food after evaluating the performance of the primers.

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*Corresponding author: Tel. +82-070-7135-9637, E-mail. csjang@kangwon.ac.kr