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Classification of plant *orostachys* used chloroplast genome through Real-Time PCR

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[Introduction]

Orostachys has been used as a health-enhancing food product in Korea. However, several *Orostachys* are classified as non-edible species. Therefore we tried to classification between edible plants and non-edible plants. Here, we have been developed a species specific primer sets using the chloroplast genome to classify seven plants such as *Orostachys japonica*, *Orostachys iwarenge*, *Orostachys malacophyullus*, *Orostachys latiellipticus*, *Orostachys margaritifolius*, *Orostachys minuta*, *Orostachys ramosus*.

[Materials and Methods]

Seven plants were purchased botanical garden. 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 Qubit® 2.0 Fluorometer (Invitrogen, Life Technologies, Grand Island, NY, USA). *Orostachys* 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]

As a result alignments of the chloroplast gene such as *matK*, *trnT-L*, *trnS-G*, *ndhF*, we are confirmed the similarity of chloroplast sequences (between *O. iwarenge* and *O. malacophyullus*, between *O. latiellipticus* and *O. ramosus*). For develop species-specific primers, we used chloroplast genes such as *matK*, *trnT-L*, *trnS-G*, *ndhF*. 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 ($R^2 > 0.99$) were obtained between the crossing point values and log DNA concentration. The Cq value difference has 4 cycle or more at non-target species (10 ng) than target species Cq values (10 pg). We developed species-specific primer sets that it could be as a useful tool to distinguish different species.

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