Exploration of the origin of nicotine in tea plant (*Camellia sinensis* L.)

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Summary

Nicotine, an alkaloid compound, is included mainly solanaceous plant such as tobacco plant (*Nicotiana tabacum*). Recently, nicotine has been detected in made tea at extremely low level, comparing with tobacco leaves. However, it is still unclear whether nicotine in tea leaves is derived from endogenous or exogenous origin. To examine the origin, we analyzed nicotine contents of many kinds of tea leaves (different parts, varieties, production area, cultivation methods and manufacturing processes), and attempted to isolate the key gene in nicotine biosynthetic pathway. Nicotine contents of tea leaves were higher in *Camellia sinensis* var. assamica than *C. sinensis* var. sinensis, but their contents were not affected during all of green, oolong and black tea manufacturing processes. On the other hand, *putrescine N-methyltransferase* (*PMT*) ortholog gene, which is known as a responsible for the biosynthesis of tropane and nicotine in tobacco roots, was detected in tea roots. These results suggested that nicotine in the tea leaves might be derived from endogenous origin.

Introduction

Nicotine has been used for a long time as a pesticide, which is thought to be help to plant defenses elicited by herbivore and pathogen attack. In 2003, the Food Sanitation Law in Japan was amended, regarding pesticide residues in foods have been introduced “Positive List System”. Accordingly, the maximum residue limit of nicotine was set to less than 0.01 μg g^{-1} DW. However, it has been reported that nicotine was detected in the made tea of more than 1.7 μg g^{-1} DW (Sheen 1988; Davis et al. 1991; Siegmund et al. 1999). Since this value greatly exceeds over the maximum residue limits of nicotine, if it is exogenous, then not only Japan but also world’s tea industry will be subjected to serious damage. On the other hand, the nicotine contents detected between varieties are greatly differences (Siegmund et al. 1999). The objective of this study was to determine the origin existing nicotine, and to isolate the key gene involved in a nicotine biosynthetic pathway in tea plant.

Materials and methods

We measured the nicotine content of each sample as follows: i) each parts (roots, stems and leaves) of the three cultivars (Yabukita, Benifuji and Benihomare, 27-years old mature tea plants) collected from in Shizuoka Tea Research Center in Kikugawa-city, Shizuoka; ii) 88 made tea produced in different regions purchased from the tea company; iii) one-year cutting rooted tea plants (cv. Yabukita) grown under hydroponics; iv) suspension cultured tea cells (derived from cv. Yabukita anther); v) products in the manufacturing processes, such as sencha, heavily steamed sencha, pan fired sencha, oolong tea and black tea. All samples were frozen, frozen-dried and ground into a fine powder. The nicotine contents were analyzed using a gas chromatography–mass spectroscopy (JOEL GCMate, USA).

Total RNA was extracted by CTAB method from tea root-tips of one-year rooted cuttings tea plants. To explore of the nicotine biosynthetic gene in tea plant, we attempted to isolate the *putrescine N-methyltransferase* (*PMT*) ortholog gene of the *N. tabacum* by using degenerated PCR method. The degenerate primers were designed from the already determined PMT amino acid sequences obtained from the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/). Sequence analysis was performed using the CEQ2000 Genetic Analysis System (Beckman Coulter, California, USA). The obtained sequences were searched against the entire GenBank database for DNA sequence (BLASTN) and protein cording (BLASTX) features. The amino acid sequence of PMT from *C. sinensis* and *N. tabacum* were aligned using the program CLASTALW tool.

Results and discussion

Nicotine was detected in all parts of all tea varieties less than 0.15 μg g^{-1} DW (Fig. 1). Furthermore, even though the growth medium does not use nicotine, nicotine was detected in the one-year rooted cuttings tea plant grown under hydroponics and the suspension cultured tea cells (data not shown). These results suggested the possibility that tea plants have nicotine biosynthesis *in vivo*.

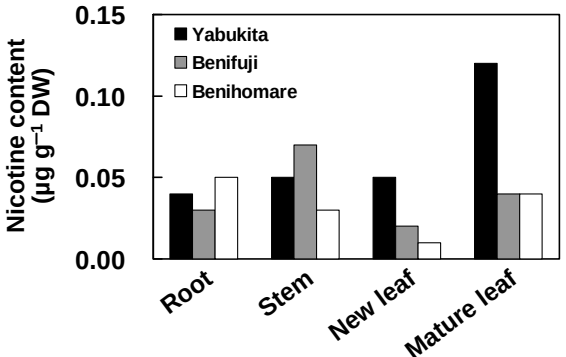

Fig. 1 Nicotine contents in different parts of the three tea cultivars.

On the other hand, nicotine contents of tea products in different regions, the black tea from Assam and Darjeeling, were higher than the green tea from Japan and China (Fig. 2). This result suggested that there was a difference in nicotine contents between varieties or production area. As measured nicotine contents of the manufacturing processes, nicotine contents of tea leaves in each processes were no change in any process of all 5 tea types (data not shown).

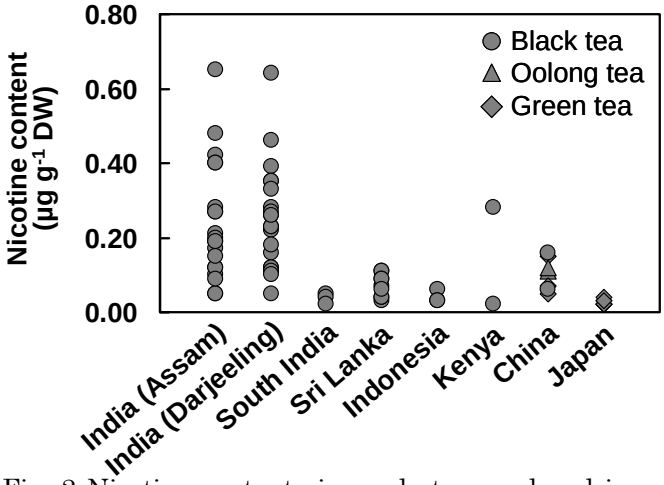

Fig. 2 Nicotine contents in made tea produced in different regions.

The amino acid sequence obtained by degenerated PCR showed highly homologue to *N. tabacum* PMT (97%) (Fig. 3). This is suggested that tea plant *PMT* ortholog gene involved in biosynthesis of nicotine.

Based on the above results, it was concluded that the nicotine detected in the tea leaves might be derived from endogenous origin.

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C. sinensis  ------------------------------------------------------------
N. tabacum   MEVISTNTNGSTIFKNGAIPMNGHQNGTSEHLNGYQNGTSKHQNGHQNGTFEHRNGHQNG

C. sinensis  ------------------------------------------------------------
N. tabacum   TSEQQNGTISHDNGNELLGSSDSIKPGWFSEFSALWPGEAFSLKVEKLLFQGKSDYQDVM

C. sinensis  ----------------------------------------------------GFTLFEML
N. tabacum   LFESATYGKVLTLDGAIQHTENGGFPYTEMIVHLPLGSIPNPKKVLIIGGGIGFTLFEML
                                                               ********

C. sinensis  RYP----------------TSRKFFPYLAANFNDPRVTLVLGDGAAFVKAAQAGYYDAII
N. tabacum   RYPSIEKIDIVEIDDVVVDVSRKFFPYLAANFNDPRVTLVLGDGAAFVKAAQAGYYDAII
             ***               .****************************************

C. sinensis  VDSSDPIGPAKDLFERPFFEAVAKALRPGGVVCTQAESIWLHMHIIKQIIANCRQVFK--
N. tabacum   VDSSDPIGPAKDLFERPFFEAVAKALRPGGVVCTQAESIWLHMHIIKQIIANCRQVFKGS
             ************************************************************

C. sinensis  ------------------------------------------------------------
N. tabacum   VNYAWTTAPTYPTGVIGYMLCSTEGPEVDFKNPVNPIDKETTQVKSKLGPLKFYNSDIHK

C. sinensis  ---------------
N. tabacum   AAFILPSFARSMIES
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Fig. 3 Alignment of amino acid sequences of *C. sinensis* of PCR fragment and *N. tabacum* PMT.

References

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