Stable nitrogen isotope analysis of chlorophylls by gas chromatography-isotope ratio mass spectrometry

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Compound-specific H, C and N isotope analyses by gas chromatography-isotope ratio mass spectrometry (GC/IRMS) have widely been used for various studies, because it allows a rapid and precise isotope analysis of specific molecules of a small amount (nanomolar amount of the element) in complex mixture. However, its application for tetrapyrrole pigments (i.e. chlorophylls) is limited due to their high molecular weight and conjugated structures. In this study, we developed a method to determine nitrogen isotopic composition ($\delta^{15}N$) of chlorophylls, by the use of chemical degradation treatment prior to the isotope analysis.

Chlorophylls were isolated by high-performance liquid chromatography. The isolated chlorophylls were transformed to pyropheophorbide derivatives by HCl treatment, and subsequently degraded to pyrrole units (maleimides) by chromic acid oxidation (Fig. 1). These chemical degradations have no substantial isotopic fractionation for chlorophyll nitrogen, and thus $\delta^{15}N$ values of the pyrrole units can be determided precisely by GC/IRMS. Analytical error (1σ, standard deviation) of the isotope measurement was always better than ±0.5‰, with the minimum sample amout of 15ngN.

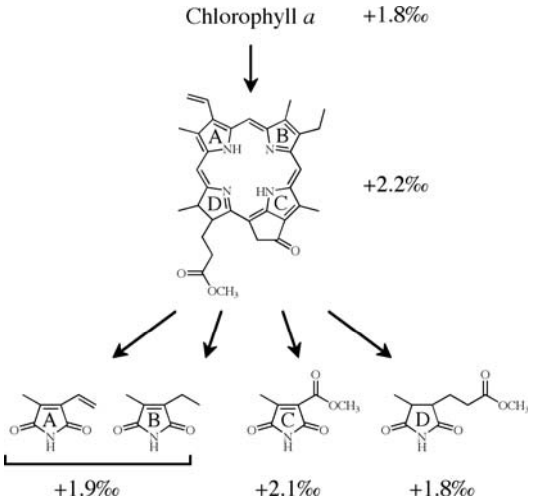

Fig. 1. $\delta^{15}N$ values of the pyrrole units from chlorophyll *a* in *Z. mays*. The $\delta^{15}N$ values of chlorophyll *a* (+1.8‰) and pyropheophorbide *a* methyl ester (+2.2‰) are determined by Flash elemental analyzer-IRMS.