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論文内容の要旨

For a decade, metabolomics approach has been developed for studies in plant science. The goal of plant metabolomics is to analyze, qualify and quantify small molecules (or metabolites) in plant tissues comprehensively. Metabolomics information from diverse plant systems will assist better understanding of physiological and developmental processes in plants. This thesis is mainly divided into three parts, in which mass spectrometry (MS) in combination with chromatography was utilized as a main tool in the metabolomics for studies in plant biology.

In Chapter 2, the analytical and data processing methods for metabolic fingerprinting and profiling by gas chromatography combined with MS (GC/MS) were established. Metabolic fingerprinting by GC/MS provides the rapid chromatographic comparison. Metabolic profiling by GC/MS allows identification and quantification of a wide range of compound classes. The established methods were applied to observe the differences between primary metabolites extracted from the leaves of *Arabidopsis* and those from *Arabidopsis* suspension cultured cells (cell line T87). The results suggested that cell suspension cultures and *Arabidopsis* leaves showed similarities in the common primary metabolite profiles, but quantitatively differences. Glucose, fructose, sucrose, malate, citrate, valine, and 4-aminobutyrate were significantly higher in the cultured cells, whereas only putrescine, threonine, and serine were higher in leaf tissues. In order to study regulations in plant metabolism comprehensively, the utilization of intact plants is more appropriate than cultured cells.

In Chapter 3, metabolic profiling was demonstrated as a useful tool to plot a specific biochemical pathway under a regulation of phyA during *Arabidopsis* seedlings de-etiolation. Etiolated *Arabidopsis* wide-type (WT) and *phyA* mutant (*phyA-211*) seedlings were irradiated with far-red light (FR), or white light (W). Primary metabolites of the treated seedlings were profiled by GC/time-of-flight-MS (GC/TOF-MS). Comparison of metabolite levels between *phyA-211* and WT seedlings under FR revealed several metabolites, which were found to be less abundant in WT than in *phyA-211* upon FR treatment, including some amino acids, organic acids, major sugars, as well as putrescine. With this comprehensive metabolite analysis, the polyamine biosynthesis was specified and investigated. The regulation of polyamine biosynthesis related genes by phyA was investigated using quantitative real-time RT-PCR. The gene expression profiles revealed that arginine decarboxylase 2 (*ADC2*) gene was much less transcribed in the WT than in *phyA-211* mutant in response to FR irradiation, suggesting that *ADC2* is involved in phyA-mediated responses during photomorphogenesis. In addition, *S*-adenosylmethionine decarboxylase 2 and 4 (*SAMDC2* and *SAMDC4*) genes are also found to be involved in phyA-mediated response.

In Chapter 4, metabolic profiling by GC/TOF-MS and capillary electrophoresis/electrospray ionization-MS/MS (CE-ESI-MS/MS) were used to study phy-regulation of primary metabolism in rice plants by the utilization of *phy* triple mutant of rice, *phyA phyB phyC* triple mutant. Principal component analysis (PCA) revealed age differentiation between young and mature leaves (YL and ML), and mutational differentiation between *phyA phyB phyC* triple mutants and the WT. Interestingly, it was obviously seen that YL of *phyA phyB phyC* triple mutant contained remarkably higher levels of glucose, fructose, galactose, and sugar phosphates than other samples. These data indicate that the lack of phyA, phyB and phyC has a significant impact on the carbohydrate metabolism and/or translocation of carbohydrates from rice young leaves. Irregular carbohydrate metabolism and sugar translocation may affect plant growth and development, resulting in the abnormal plant architecture. I proposed that sugar metabolism, carbon partition, and sugar transportation are impaired in the *phyA phyB phyC* triple mutants, particularly in the source organs.

In the work underlying this thesis, I have established GC/MS based metabolic fingerprinting and profiling. By using metabolic profiling by GC/MS systems, primary metabolites can be examined in high-throughput fashion with good reliability and robustness. Metabolic profiling by GC/MS facilitates the investigation of metabolic changes associated with the loss of phy functions. The metabolic profiles from GC/MS system proposed the important roles of phys in the regulation of primary metabolism in *Arabidopsis* and rice plants. The combination of metabolic and gene expression profiling revealed for the first time that phyA has a role in the regulation on *Arabidopsis* polyamine biosynthesis.

論文審査の結果の要旨

本論文は，第１章「緒言」，第２章「シロイヌナズナ葉および T87 培養細胞のメタボリックフィンガープリンティングならびにメタボリックプロファイリング」，第３章「代謝プロファイリングと遺伝子発現プロファイリングの統合によって明らかとなったシロイヌナズナにおけるポリアミン生合成のフィトクロム A による制御」，第４章「イネの炭化水素代謝におけるフィトクロム制御についてのメタボロミクス」および第５章「総括」より構成されている．

第１章では，メタボロミクスという比較的新しい技術と植物のフィトクロム応答についての背景が詳しく記されており，これまで分子遺伝学的な研究が主流であったフィトクロム応答を代謝レベルで網羅的に解析する意義ならびに目的が丁寧に解説されている．第２章では，植物研究にメタボロミクスを適用するにあたり，解決すべき技術課題が解決されており， GC/MS で得られるクロマトグラムをメタボロミクスに用いるための方法の開発について記されている．第３章では，新しく開発されたメタボロミクスを用いてモデル植物であるシロイヌナズナのフィトクロム応答を解析し，ポリアミンの生合成経路中でプトレシンレベルが ADC2 の遺伝子の発現制御を介して調節されていることが新たに見出されている．第４章では，日本にとって最も重要な穀類であるイネについてより詳細にメタボロミクスを行い，フィトクロムが糖代謝の調節に重要な役割を担っていることが明らかにされている．特に，フィトクロムが糖の転流に関わっているという興味深い仮説が導かれている．第５章で植物研究におけるメタボロミクスの有用性について本研究で得られた成果を中心に記されている．

以上のように，本論文はメタボロミクスの技術開発を行い，これを植物研究に用いることでフィトクロムによって制御されている代謝経路を明らかにすることに成功している．本研究の技術や戦略は今後広くライフサイエンスならびにバイオテクノロジーに貢献すると期待出来る．

よって本論文は生物工学専攻の博士論文として価値あるものと認める．