

Iron Homeostasis and Metabolic Alteration in *Hyoscyamus albus* Roots under Iron Deficiency

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Iron (Fe) is an essential micronutrient for all organisms including plants. In plants, Fe is involved in a wide range of redox reactions, such as those of the electron transport chains of photosynthesis and respiration. Fe paucity is one of the major nutrient constraints for plant growth and development in the area of calcareous soils where plants stand on, owing to the extremely low availability of Fe. Such nutrient deficiency problem also directly affects to human health as plants provide all essential nutrients for human.

Hyoscyamus albus is a well-known source of tropane alkaloids, hyoscyamine, and scopolamine, which are biosynthesized in roots. Previously, it was found in my laboratory that, *H. albus* roots were able to grow under Fe deficiency, and secrete flavin (riboflavin) in the rhizosphere. Furthermore, feeding experiments with mitochondrial component specific inhibitors have indicated that mitochondrial electron transport chains (mtETC) involving in aerobic respiration changes to more energy-efficient and iron-saving manner under Fe deficiency, and suggested that riboflavin secretion occurs as a result of the underuse of flavoprotein complexes I and/or II.

This energy-efficient and iron-saving manner is likely to be a more extensive phenomenon involving wide-ranging adaptations at the cellular and tissue levels to maintain Fe homeostasis. To explore this idea, I have conducted a global protein expression survey using a proteomic approach. In addition, I have determined the metabolic changes, including flavin secretion and tropane alkaloid production, from a biochemical point of view under Fe-suboptimal condition as follows:

Chapter 2: Metabolic alteration induced by Fe deficiency

In this chapter, I focused on the relationship between riboflavin production and respiration activity. In addition, alkaloid production and organic acid production by *H. albus* roots were determined under Fe deficiency. The results revealed that FMN hydrolase contributed to riboflavin secretion and also suggested that such secretion possibly linked with the enhancement of respiratory activity. To investigate the effects of Fe deficiency on alkaloid biosynthesis, gene expression studies were undertaken both for *H6H* and *Cyp80F1*, which are involved in the tropane alkaloid biosynthesis and require Fe as a cofactor for their activities. In addition, tropane alkaloid contents were determined. Reduced gene expression was observed in the case of both of these proteins and was accompanied by a decrease in the contents of hyoscyamine and scopolamine under Fe deficiency. It was also observed that *H. albus* roots have secreted higher amounts of malate than citrate in the rhizosphere under Fe-suboptimal conditions.

Chapter 3: Establishment of a small-scale proteomics for *H. albus* root tips

I had started to investigate the holistic view of the biochemical changes by a proteomic approach. But, proteomics of *H. albus* roots were challenging due to their recalcitrant tissues with abundant interfering materials that affected two-dimensional gel electrophoresis (2DE) and mass spectrometry (MS). For overcoming such problems, I established an efficient protein extraction protocol for *H. albus* roots using bead-beating disruption with PVPP and DTT, and acid guanidinium-phenol-chloroform extraction methods that allowed high-quality 2DE, high-sensitive MALDI-MS, and cross-species protein identification.

Chapter 4: Survival strategy under Fe deficiency shown by proteomics

Using an established small-scale proteomic approach, comparative proteomics has been done using proteins from root tips cultured with and without Fe. The protein profiles showed that under Fe deficiency, most of the proteins involved in carbohydrate metabolism were up-regulated, but a subset of mitochondrial NAD-dependent malate dehydrogenases was down-regulated, possibly resulting in malate secretion. A significant decrease was also observed in the levels of hyoscyamine 6 β -hydroxylase (H6H), which requires Fe and is involved in the conversion of hyoscyamine to scopolamine. I also observed down-regulation of the expression of mitochondrial respiratory complex I (NADH dehydrogenase Fe-S protein 1) which was consistent with previous result obtained by feeding experiments with mitochondrial component-specific inhibitors.

Chapter 5: Uptake of heavy metals by various plants growing in Bangladesh

To understand the uptake ability of heavy metals, including Fe, heavy metal accumulations were analysed in the plants growing on various soils in Bangladesh. The results showed that leaves of some plants accumulated higher amounts of arsenic. Such plants contained less amounts of Fe in comparison to plants which grew in the soils containing less amounts of arsenic.

Chapter 6: Concluding remarks

Under Fe deficiency, our data support the conclusion that the proteins necessary for remodeling of root development seem to be derived from their amino acids through proteolysis, rather than through *de novo* biosynthesis. Repressions of the Fe or ATP required proteins must ensure the balanced utilization of Fe and energy to maintain cellular processes linked with Fe homeostasis under Fe-deficient conditions.

