Development of copper-inducible gene expression system for higher plants and its application study

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Background and Objectives

Copper is an essential element for all known living organisms, including humans and other animals. Though necessary at low concentrations, it is potentially toxic at higher levels. To develop a simple monitoring tool for copper content in environment, I focused on copper-inducible reporter gene expression system that is based on regulation of the yeast metallothionein gene. To date, this system has rarely been used to control the expression of genes of interest, probably due to the inefficiency of the gene expression systems. I decided to start with improving this system to achieve high levels of reporter gene expression in a dose-dependent manner so that it can be detected easily, and to maintain the basal expression to negligible level in the absence of copper, for preventing false positive. After well characterizing a newly developed system, I evaluated for the potential use as a monitoring tool of bioavailable copper. In this dissertation, I describe key points I found during development of an improved system, and demonstrate its potential application to detect bioavailable copper in soil.

Chapter 1:　Development of an improved copper-inducible gene expression system

In this chapter, I showed an improved system can be applied in the field and can be regulated at approximately one-hundredth of the rate used for registered copper-based fungicides. In the presence of copper, a translational fusion of the ACE1 transcription factor with the VP16 activation domain (VP16AD) of herpes simplex virus strongly activated transcription of the *GFP* gene in transgenic *Arabidopsis*. Interestingly, insertion of the *To71* sequence, a 5′-untranslated region of the *130k/180k* gene of tomato mosaic virus, upstream of the *GFP* gene reduced the basal expression of *GFP* in the absence of copper to almost negligible levels, even in soil-grown plants that were supplemented with ordinary liquid nutrients. Exposure of plants to 100 μM copper resulted in an over 1,000-fold induction ratio at the transcriptional level of *GFP*. This induction was copper-specific and dose-dependent with rapid and reversible responses. Thus, the newly developed copper-inducible gene expression system showed good performance in a tightly regulated and highly responsive manner.

Chapter 2: Application study to control of flowering time

If the system is well improved, it can be useful not only as a monitoring tool, but also in various fields such as the efficient production of value-added proteins or metabolites in plant culture cells, physiological regulation of plant, analysis of gene function, and phytoremediation. To validate the performance of the newly developed system, among these potential applications, I attempted and succeeded in regulating floral transition by copper treatment.

Chapter 3: Application study to a new detection tool for bioavailable copper in soil

Currently, only total copper concentration in soil is normally measured and used as a regulatory standard despite bioavailable copper in soil being limited due to absorption by soil components. Thus, it is important to distinguish between total copper concentration and bioavailable copper concentration. Unfortunately, a simple, convenient, and beneficial tool for detection of bioavailable copper is lacking. In this chapter, I evaluated some transgenic plants carrying the newly developed copper-inducible *GFP* gene expression system, by transferring them to soil supplemented with various amounts of copper. When supplemented with $CuSO_4$ solution, GFP fluorescence was observed at 0.4 parts per million (ppm) or more, but when supplemented with metallic copper, more than 20 ppm was required for GFP fluorescence. These results indicated that uptake of copper when supplemented with $CuSO_4$ solution is 50 times easier than that when supplemented with metallic copper, and that the transgenic plants respond selectively to bioavailable copper. Sixty independent transgenic plants were generated with different copper detection sensitivity. Thus, the transgenic plant with the optimal target concentration range of bioavailable copper for the intended use can be chosen.

Concluding Remarks

This study successfully demonstrates a new transgenic plant-based system is able to detect bioavailable copper without any extraction and purification steps. In addition, these results indicate that the newly developed copper-inducible system can accelerate gene functional analysis in model plants and can be used to generate novel agronomic traits in crop species.