P14 Isolation and Expression of *Lai* Genes Encoding α-amylase Inhibitor from *Lablab purpureus*

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Objectives

Aspergillus flavus is a fungal pathogen of maize causing an important ear rot disease when plants are exposed to drought and heat stress. Our previous research has suggested that α-amylase of A. flavus promotes aflatoxin production in the endosperm of infected maize kernels. We also reported that the 36-kDa α-amylase inhibitor purified from *Lablab purpureus* (AILP) inhibits the α-amylase from a number of fungi but not those from animal and plant sources. Partial peptide sequence of the AILP indicated that AILP is similar to lectin members of a lectin-arcelin- α-amylase inhibitor family described in common bean and shown to be a component of plant resistance to insect pests. The expression of AILP in maize will may result in resistance to *A. flavus* and reduce aflatoxin contamination in maize. To test this hypothesis, the way of approach is the cloning of the gene encoding AILP and expression of the AILP gene to text α-amylase inhibitor activity.

Materials and Methods

1. Materials: *Lablab purpureus*, *Aspergillus flavus*
2. Methods: PCR, Genomic walker PCR, Genomic Southern, Western analysis

Result and Discussion

A fragment of the gene that encoded for 82 residues of AILP amplified with degenerated primers based on peptide sequencing of AILP. Blasting the deduced sequence in database revealed that the peptide is highly homologous to several lectins. Genomic Southern analysis with the PCR product as a probe indicates that *Lai* (*Lablab purpureus* α-amylase inhibitor) gene is a multigenic family. To get a complete coding region of *Lai* genes, genomic walker PCR was performed. Herein, we isolated six different *Lai* genes encoding α-amylase inhibitor. The complete coding region of *Lai* genes was subcloned into *E. coli* overexpression vector pET28. The AILP overproduced in pET28 was insoluble protein. We are tying to get a soluble protein in the *E.coli* strains using different conditions known to get a soluble protein. At the same time, yeast strains carrying AILP in yeast expression vector, pESC or pPICZalpha, is under construction.

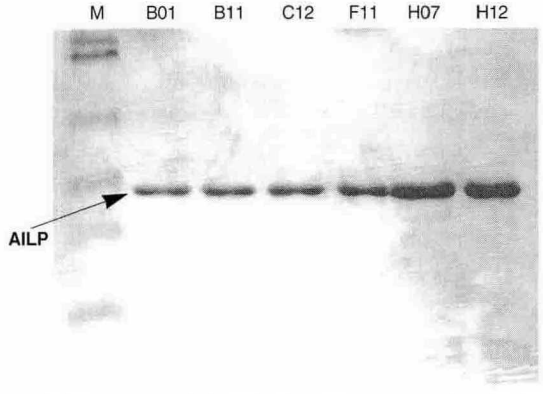

Figure 1. Western analysis. His Tag- AILP overproduced in *E.Coli* was detected by His Tag antibody.

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