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Functional characterization of a lectin-arcelin-α-amylase inhibitor family in *Lablab purpureus*

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Objectives

To characterize of *Lai* genes encoding α-amylase inhibitor (AILP) that inhibits growth of *A. flavus* and fungal α-amylase

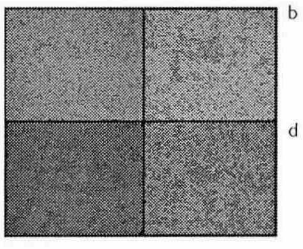
Materials and Methods

1. Materials : *Pichia pastoris, Aspergillus flavus, Lablab purpureus*
2. Methods : Genome Walker PCR, Methanol induction, Lectin activity assay, α-amylase & α-amylase inhibitor assay, Western blot analysis

Results and Disscusion

Aspergillus flavus is a fungal pathogen of maize causing an important ear rot disease when plants are exposed to drought and heat stress. Associated with the disease is the production of aflatoxins. It is reported that α-amylase of *A. flavus* promotes aflatoxin production in infected maize kernels and the 36kDa α-amylase inhibitor purified from *Lablab purpureus* (AILP) inhibits the α-amylase of *A. flavus*. AILP also showed lectin activity. Partial peptide sequence of the AILP indicated that AILP is a lectin-arcelin-α-amylase inhibitor family. Herein, six different *Lai* (*Lablab purpureus* α-amylase inhibitor) genes isolated and characterized. To examine which protein(s) encoded *Lai* genes have both lectin and α-amylase inhibitor activities of AILP, the coding region without signal peptide of *Lai* genes was subcloned into pPICZαA vector system secreted recombinant protein into culture medium of *P. pastoris*. We tested a lectin activities (hemagglutination) using five recombinant proteins. Four of them agglutinated human red blood cells (RBC). Three recombinant proteins having lectin activity has α-amylase inhibitor activity. These data show that three among recombinant proteins characterized here were bifunctional protein having lectin and α-amylase inhibitor activities.

A



B

Isoform of α-amylase inhibitor	Inhibition (%)
pPICZ-B11	44
pPICZ-C12	25
pPICZ-H07	41

Fig. 1. A Lectin activity, (a)control (b)B11(8μg), (c)C12, (d)H07 (4μg) proteins agglutinated papain-treated RBC. B Inhibition rate of α-amylase inhibitor, 35U α-amylase was incubated with 2nmole α-amylase inhibitor. The amount of reducing sugars released was determined with Nelson&Somogyi method.

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