

Functional analysis of the expressed product of a rice allergen cDNA in *E. coli*

As a part of plant defense system, the proteinous inhibitors of α -amylase are grouped into the cereal α -amylase/trypsin inhibitor family and have been found widely existing in most of the cereals plants, e.g. wheat, sorghum, and maize. Rice was reported to be an exception, lacking α -amylase inhibitors. In recent years, proteins called rice allergen (about 14–16 kD^[1]) were isolated from rice endosperm and found to be encoded by a multigene family^[3]. Several rice allergen (RA) cDNAs have been cloned and grouped into the α -amylase/trypsin inhibitor gene family through sequence comparison. However, it is still unclear whether the RA proteins inhibit α -amylase or inhibit trypsin or even both.

A rice allergen gene, RA17^[2], was amplified and cloned from rice immature seeds by RT-PCR. This gene was then cloned into the prokaryotic expression vector pBV220 (recombinant designated pBVR08) and a 18 kD protein, as expected, was expressed in *E. coli* after transformation and induction. Crude proteins were extracted and the inhibitory activities towards insect α -amylase, bovine trypsin and growth of the larvae of *Heliothis virescens*, one of the most devastative pests in cotton, were determined.

Enzyme activity inhibitory experiments show that the inhibitory activity of the expressed products of pBVR08 is more than 50 % toward insect α -amylase (fig. 1) and no significant effect toward bovine trypsin was found (data not shown), suggesting that the expressed products of pBVR08 can strongly inhibit the activity of α -amylase *in vitro* but lack the inhibitory activity toward bovine trypsin. Larvae-feeding experiment shows that the mean weight of larvae in the group fed by forage with the expressed products of pBVR08 is obviously lower than that of the two controls (fig. 2). This result indicates that the expressed products of pBVR08 strongly inhibit the development of larvae of *H. virescens*, which is the first report on the potential of inhibiting the development of larvae of *Lepidoptera* by a cereal α -amylase/trypsin inhibitor.

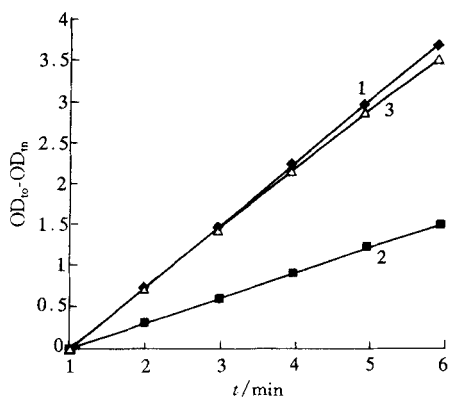


Fig. 1. Assay on inhibitory activity of crude proteins containing pBVR08 expression product towards α -amylase of *Tenebrio molitor*. 1, α -amylase only; 2, α -amylase with crude proteins containing pBVR08 product; 3, α -amylase with crude proteins without pBVR08 product.

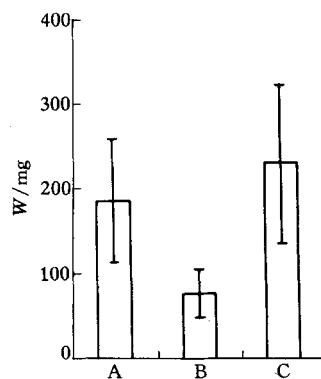


Fig. 2. Assay on anti-insect ability of pBVR08 expression product. A, Normal forage; B, normal forage with crude proteins containing pBVR08 product; C, normal forage with crude proteins without pBVR08 product.

References

- Chen, Z., Qu, L. J., Gu, H. Y. *et al.*, Studies on allergens in higher plants, *Plant Physiology Communications* (in Chinese), 1997, 33(1): 56.
- Adachi, T., Izumi, H., Yamada, T. *et al.*, Gene structure and expression of rice seed allergenic protein belonging to the α -amylase/trypsin inhibitor family, *Plant Mol. Biol.*, 1993, 21: 239.
- Alvarez, A. M., Adachi, T., Nakase, M. *et al.*, Classification of rice allergenic protein cDNAs belonging to the α -amylase/trypsin inhibitor gene family, *Biochim. Biophys. Acta*, 1995, 1251: 201.

JIN Jianhua¹, ZHANG Houbin¹, QU Lijia¹, XIE Ming¹, GU Hongya¹ and CHEN Zhangliang^{1, 2}

1. State Key Laboratory of Protein Engineering and Plant Genetic Engineering, Peking University, Beijing 100871, China; 2. De Montfort University, The Gateway, Leicester LE1 9BH, UK