Short Communication

Identification of the Growth Inhibitor on Azuki Bean Weevil in Kidney Bean (*Phaseolus vulgaris* L.)

Masao ISHIMOTO and Keisuke KITAMURA

*Legume Breeding Laboratory, National Agriculture
Research Center, Tsukuba, Ibaraki, 〒 305*

Kidney beans contain a water-soluble substance which strongly inhibits the larval growth of azuki bean weevil. The substance has been purified by fractionation methods involving dialysis, precipitation with ammonium sulfate, and chromatographies with DEAE-Sephacel and Con A-Sepharose. The inhibitory activity was bioassayed using the artificial beans incorporating the fractionated materials after lyophilizing into the azuki bean meal. The purified substance, which is one of glycoproteins having the molecular weight of about 100,000, showed inhibitory activity on α -amylase. When the α -amylase inhibitor was incorporated at 0.2-0.5% into the artificial beans, the larvae of the weevil could not develop by feeding them and died. The results obtained here show that the α -amylase inhibitor is responsible for the protection of kidney beans against the attack of azuki bean weevil.

KEY WORDS : *Phaseolus vulgaris* L., kidney bean, *Callosobruchus chinensis* L., azuki bean weevil, α -amylase inhibitor.

Introduction

The azuki bean weevil (*Callosobruchus chinensis* L.) is commonly known as a pest of azuki bean (*Vigna angularis*) and mung bean (*V. radiata*) during storage. The weevil larvae can be raised also on other legume seeds such as pea (*Pisum sativum*) and soybean (*Glycine max*). But they can not be raised on kidney bean (*Phaseolus vulgaris*) and black gram (*Vigna mungo*). ISHII (1952) reported that there exist certain growth inhibitory substances in kidney beans, which can be extracted with water. None of these water-soluble compounds inhibiting the weevil growth has been identified until today.

Materials and Methods

The seeds of two kidney bean varieties, Taishou kintoki and Shiromi shakugosun were used. The growth inhibitory substance was extracted with 20 fold of 0.02 M sodium phosphate buffer (pH 6.7) from each kidney bean meal. The purification procedure for the substance is detailed in results and discussion.

The inhibitory activity was bioassayed using the artificial beans (ISHII, 1952) incorporated the fractionated materials after lyophilizing into the azuki bean meal at varying concentrations. The artificial beans on the glass dishes were kept in a chamber at 30°C and 70% relative humidity for at least two weeks after initial oviposition. The larval growth was examined, and the concentration of each fraction at which the larvae died before the second instar was recorded.

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Results and Discussion

The growth inhibitory substance was not passed through a dialyzing cellulose tube, and concentrated by the precipitation with ammonium sulfate from 20% to 60% saturation. The precipitate was dialyzed against water and lyophilized. All the first instar larvae died just after boring into the artificial beans containing 5%(w/w) of the lyophilized material. The inhibitory activity of the material was lost after a treatment with of 1/50 (w/w) protease (Sigma protease from bovine pancreas crude Type I). The results indicate that the growth inhibitory substance might be protein.

On the Sephacryl S-200 column the protein eluted at almost the same fraction as that of the lectin, which has the molecular weight of about 126,000 (LIENER, 1976). The Con A-Sepharose-absorbed fraction having the strong inhibitory activity on the larval growth of the weevil was applied to the column of DEAE-Sephacel, and bound proteins were recovered by a gradient elution (0.02–0.25 M) of sodium phosphate buffer (pH 6.7). The elution profile was shown in Fig. 1. It was found that the first peak had hemagglutination activity tested by serial dilution of rabbit erythrocytes and the second peak had inhibitory activity on the α -amylase of porcine pancreas (Sigma Type I-a) and amylase activity in weevil larvae homogenate, and that the second is much more toxic to the weevil larvae than the first. JANZEN *et al.* (1976) reached a conclusion that the lectin in kidney bean was responsible for protecting them from the attack by *Callosobruchus maculatus*, because the larvae could not grow in the artificial beans containing 5% lectin. In our experiment, however, the lectin preparations from kidney beans did not affected *C. chinensis* larvae at that level. One reason of this disagreement may be due to the difference of the insect species tested. Another may be that their lectin preparations were contaminated with the α -amylase inhibitor.

The second peak fraction was rechromatographed on the DEAE Sephacel column. The

0.02M Sodium phosphate buffer (pH6.7) 0.25M
Fig. 1. DEAE-Sephacel chromatography of Con A-Sepharose-absorbed fraction of Taishou kintoki, and inhibitory activities (Δ) of fractions against α -amylase from porcine pancreas using a modified method of GATEHOUSE *et al.* (1986).

Fig. 2. SDS-PAGE analysis of α -amylase inhibitors. (A) Shiromi shakugosun ; (B) Taishou kintoki.

single major peak which showed the strong inhibitory effect on α -amylase activity was recovered. The purified α -amylase inhibitor gave a single protein band on a native polyacrylamide gel electrophoresis (PAGE) gel, and 3-4 bands with a mol. wt. of 15,000-20,000 on a sodium dodecyl sulfate (SDS)-PAGE gel (Fig. 2). The α -amylase inhibitor was highly toxic to the larvae: all the weevil larvae died before the second instar in the artificial beans incorporated the protein at 0.2-0.5%. MARSHALL and LAUDA (1975) reported that the α -amylase inhibitor was present at a level of 0.4-0.5% in kidney bean seeds. Thus, the level of the α -amylase inhibitor in the seeds seems to be enough to protect them against the attack of azuki bean weevil.

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インゲンマメ種子に含まれるアズキゾウムシ生育阻害物質の同定

石本 政男・喜多村啓介

(農業研究センター・つくば市観音台, 〒 305)

アズキゾウムシはアズキ, リョクトウ, ササゲ種子を食害する貯蔵害虫であり, 我が国においてはアズキ貯蔵中の最大の害虫である. 栽培アズキには本虫に対する抵抗性は見出されていない. アズキゾウムシは, 寄主としていないダイズ, ソラマメにおいても生育が可能であるが, インゲンでは生育できない. また, インゲン種子中には本虫に対して生育阻害活性を有する水溶性物質があることが明らかにされている (ISHII, 1952). そこで, インゲン種子に含まれる水溶性の生育阻害物質の単離・同定を試みた.

インゲン種子粉の 0.02 M 磷酸緩衝液 (pH 6.7) 抽出物を分画し, 水に対して透析後, 凍結乾燥し, 種々の割合でアズキ粉に混合した人工豆を作り, どの画分に生育阻害物質が濃縮されるかを調べ, 純化を進めた.

磷酸緩衝液により抽出された生育阻害物質は, 20~60% 飽和の硫酸沈澱画分に濃縮され, プロテアーゼに感受性であった. さらに, 生育阻害物質は Con A-Sepharose に吸着し, Sephacryl S-200 によるゲルろ過でレクチン (分子量約 12,600) とほぼ同じ位置に溶出した. 生育阻害物質は, Con A-Sepharose に吸着後, DEAE-Sephacel によるイオン交換クロマト (0.02~0.25 M 磷酸緩衝液 (pH 6.7)) で, レクチンの後に溶出した (Fig. 1). 単離された生育阻害物質は, 強い α -アミラーゼ阻害活性を有する α -アミラーゼインヒビターであり, インゲン種子中の濃度 (0.4~0.5%) でアズキ粉に混入することによりアズキゾウムシの生育を完全に阻害した. さらに高度に精製した α -アミラーゼインヒビターは, native PAGE により単一の泳動バンドを与え, SDS-PAGE により 3~4 本のバンドを与えた (Fig. 2).

以上の結果から, インゲン種子中に含まれる水溶性のアズキゾウムシ生育阻害物質は, 分子量 10 万程度の糖蛋白質である α -アミラーゼインヒビターである.