TARC Report

Trypsin and Chymotrypsin Inhibitors in Winged Bean

By JIRO ABE*, HIROSHI NAKAMURA**, HIROSHI SHIBATA***, SHIGEMI NORIOKA***, SABURO HARA*** and TOKUJI IKENAKA***

*, ** Okinawa Branch, Agriculture Research Center
(Maesato, Ishigaki, Okinawa, 907-01 Japan)
*** Department of Chemistry, College of Science, Osaka University
(Toyonaka, Osaka, 560 Japan)

Introduction

The winged bean (*Psophocarpus tetragonolobus*) is a tropical legume with a high protein and oil content.[6] Like other legumes, its ripe seeds contain several toxic substances, antinutritional factors, etc. such as lectins, hydrocyanic glycosides, protease inhibitors, and tannins.[1] The study should be done systematically to know the kinds of antinutritional factors occurring in each part of this crop, since almost all parts of the crop can be eaten raw or cooked.

Content of trypsin inhibitors in winged bean seeds has been reported to be the highest among several legume seeds.[3,4] Content of trypsin inhibitors together with that of chymotrypsin inhibitors in edible parts of the winged bean were determined. This study constitutes a part of the TARC research program "Introduction of winged bean" and was carried out jointly with biochemists of the Osaka University.

Present address:
* Tohoku National Agricultural Experiment Station
(Morioka, Iwate, 020-01 Japan)
** National Agriculture Research Center
(Yatabe, Ibaraki, 305 Japan)

Materials and methods

Three winged bean varieties, "UPS-31" introduced from Papua New Guinea, "Tpt-2" from Nigeria and "Colombia" from Colombia, and a variety, "Tpt-7", of a related species, *Psophocarpus palustris*, were sown in the field of TARC at Ishigaki Island, 24°N, early in June, 1982.

To study the relationship between fruit development and biosynthesis of trypsin and chymotrypsin inhibitors, newly formed flowers of UPS-31 and Tpt-2 were tagged. Pods of various ages, and other edible parts such as buds, flowers, leaves, stems and tubers were sampled in mid-November, 1982. The pods were immediately separated into pericarps and seeds. Tubers and ripe seeds of Colombia and Tpt-7 were also sampled and used for study.

Two to five grams of each sample were chipped and homogenized with 0.1*M* Tris-HCl buffer (pH 7.7, 10 ml per g of sample), as soon as possible after sampling. The homogenized samples were filtered through a filter paper and the filtrates were used for the assay after appropriate dilution.

Activity of trypsin and chymotrypsin inhibitors was determined spectrophotometrically according to the method of Schwert and Takenaka.[7] An aliquot of inhibitor solution

was incubated for 4 min at 30°C with 26.3 μg of bovine trypsin (twice crystallized, Type III) in a total of 2.5 ml of 0.05*M* Tris-HCl buffer (pH 8.0) containing 0.02*M* $CaCl_2$. Two milliliters of the inhibitor-enzyme mixture and 0.1 ml of 0.01*M* benzoyl-L-arginine ethyl ester (BAEE) solution in the same buffer were pipetted into a cuvette, and the change of absorbance at 253 nm was recorded against a reference cuvette containing 2 ml of the buffer and 0.1 m*l* of the substrate solution with a Hitachi recording spectrophotometer (model 320 or 220). The anti-chymotrypsin activity was determined in a similar way using bovine α-chymotrypsin (three times crystallized, Type II) and 0.02*M* acetyl-L-tyrosine ethyl ester (ATEE) solution in acetonitrile, as an enzyme and a substrate solution, respectively. The change of the absorbance was measured at 237 nm.

One unit of the inhibitory activity is defined as the amount of inhibitor which inactivates 1 mg of active enzyme. The specific activity is defined as the number of the unit per g of protein.

Protein contents of samples were measured by the method of Lowry et al.[5] using bovine serum albumin as a standard protein.

Results

Activity of trypsin and chymotrypsin inhibitors in buds, flowers, pericarps, leaves and stems was less than 0.4 unit per g of fresh weight of each part, suggesting that these parts except seeds and tubers had little problem of the protease inhibitors from the practical view point (Table 1).

The activity in tubers increased with time, showing a difference in activity of trypsin inhibitors between UPS-31 and other varieties of *P. tetragonolobus*. UPS-31 showed the highest value (Table 2). On the other hand, no appreciable varietal difference was found with chymotrypsin inhibitors, which ranged only from 10.2 to 11.4 units per g of the tubers, although the content of chymotrypsin inhibitors was two times as much as that of trypsin inhibitors.

Table 1. Trypsin and chymotrypsin inhibitors in each part of plant except tubers and seeds

		TIU[2]	CIU[3]	TSA[4]	CSA[5]
Bud					
UPS-31		0.35	0.39	2.2	2.5
Tpt-2		0.08	0.32	0.6	2.2
Flower					
UPS-31		0.03	0.11	0.2	0.9
Tpt-2		0.08	0.29	0.6	2.2
Pericarp[1]					
UPS-31	min	0.02	0.05	0.1	0.5
	max	0.09	0.12	0.9	1.7
Tpt-2	min	0.0	0.04	0.0	0.6
	max	0.31	0.24	5.5	4.2
Stem					
UPS-31	young	0.12	0.04	0.7	0.2
	old	0.09	0.07	0.6	0.5
Tpt-2	young	0.01	0.13	0.1	0.8
	old	0.0	0.02	0.0	0.1
Leaf					
UPS-31	young	0.0	0.13	0.0	0.4
	old	0.0	0.05	0.0	0.1
Tpt-2	young	0.04	0.11	0.2	0.5
	old	0.0	0.27	0.0	0.7

1) Sampled between 23 and 48 days after flowering for UPS-31, and between 13 and 39 days for Tpt-2.
2) Trypsin inhibitor unit in 1 g (fresh weight) of sample.
3) Chymotrypsin inhibitor unit in 1 g (fresh weight) of sample.
4) Specific activity of trypsin inhibitor (TIU/g of protein).
5) Specific activity of chymotrypsin inhibitor (CIU/g of protein).

Fig. 1 shows changes in activities of trypsin and chymotrypsin inhibitors in the course of seed development of UPS-31 and Tpt-2. The activities were very low in immature seeds sampled up to the 30th day after flowering, and then they began to increase rapidly. The activities (on dry weight basis) and specific activities reached the maximum about 35 and 40 days after flowering for Tpt-2 and UPS-31, respectively.

The result indicates that green pods, the most popular edible part generally harvested 15 to 20 days after flowering, are free from these protease inhibitors. As in the case of tubers, the activities of trypsin and chymotrypsin

Table 2. Trypsin and chymotrypsin inhibitors in tubers and ripe seeds

		TIU[1]	CIU[2]	TSA[3]	CSA[4]
Tuber					
UPS-31	young	0.36	3.13	2.6	22.7
	old	7.11	11.4	105	168
Tpt-2	young	0.26	0.43	3.1	5.2
	old	4.21	10.5	60.7	151
Colombia	young	0.32	1.39	3.2	14.0
	old	4.41	10.2	55.8	129
Tpt-7 (*P. palustris*)	young	0.52	0.07	6.8	0.9
Ripe seed					
UPS-31		15.5	37.5	32.4	78.5
Tpt-2		8.2	26.7	18.1	58.8
Colombia		3.0	28.1	5.8	54.8
Tpt-7		42.0	34.6	79.0	65.1

1) Trypsin inhibitor unit in 1 g (fresh weight) of sample.
2) Chymotrypsin inhibitor unit in 1 g (fresh weight) of sample.
3) Specific activity of trypsin inhibitor (TIU/g of protein).
4) Specific activity of chymotrypsin inhibitor (CIU/g of protein).

inhibitor in the ripe seeds were the highest in UPS-31 among 3 varieties used. The activity of trypsin inhibitors per g of fresh weight ranged from 3.0 (Colombia) to 15.5 units (UPS-31), while that of chymotrypsin inhibitors ranged from 26.7 to 37.5 units.

The activity of chymotrypsin inhibitors (34.6 units) of *P. palustris* (Tpt-7) was very similar to that of *P. tetragonolobus*, but that of trypsin inhibitors (42.0 units) was three times as high as that of UPS-31.

Discussion

The trypsin inhibitor is known as one of the antinutritional factors in legumes.[1,3,4] However, the chymotrypsin inhibitor has received little attention from a nutritional point of view.

The present study indicated that three varieties of *P. tetragonolobus* showed the activity of chymotrypsin inhibitors 2 to 9 times as high as that of trypsin inhibitors in ripe seeds. The former activity was less variable among varieties than the latter. On the contrary, *P. palustris* seeds showed the highest activity of the trypsin inhibitors among the legume seeds used. That activity was more than twice that of UPS-31. Furthermore, *P. palustris* showed much higher activity of trypsin inhibitors than that of chymotrypsin inhibitors in the ratio of 5 : 4 while *P. tetragonolobus* had less trypsin inhibitors than chymotrypsin inhibitors. Such a change of the ratio of the two protease inhibitors between the two species might be related with the evolutionary process of each species. It would be worth surveying a wider range of leguminous species.

The most interesting is that both the protease inhibitors begin to increase in seeds simultaneously about a month after flowering. This time is regarded as a critical stage of seed development because the seeds attain to the maximum size[2] and become germinative (unpublished data) at that time. The fact that the protease inhibitors are observed only in tubers and seeds of the winged bean seems to suggest the ecological significance of these protease inhibitors. Namely, the inhibitors may serve to protect the important organs for survival (tubers) and reproduction (seeds) against predators.

Summary

Attempt was made to determine the activity of trypsin and chymotrypsin inhibitors in edible parts of the winged bean, in which the inhibitory activities have been reported to be the highest among several legume beans.

Chymotrypsin inhibitors, which have received little attention so far as compared with trypsin inhibitors, were found to exist in seeds two to nine times as much as trypsin inhibitors, and their contents differed a little among varieties.

The result also indicated that flowers, green pods, leaves and stems were free from the protease inhibitors. Trypsin and chymotrypsin inhibitor contents simultaneously began to increase in the seeds about a month after flowering, when the seeds attained to the maximum size and became germinative. The

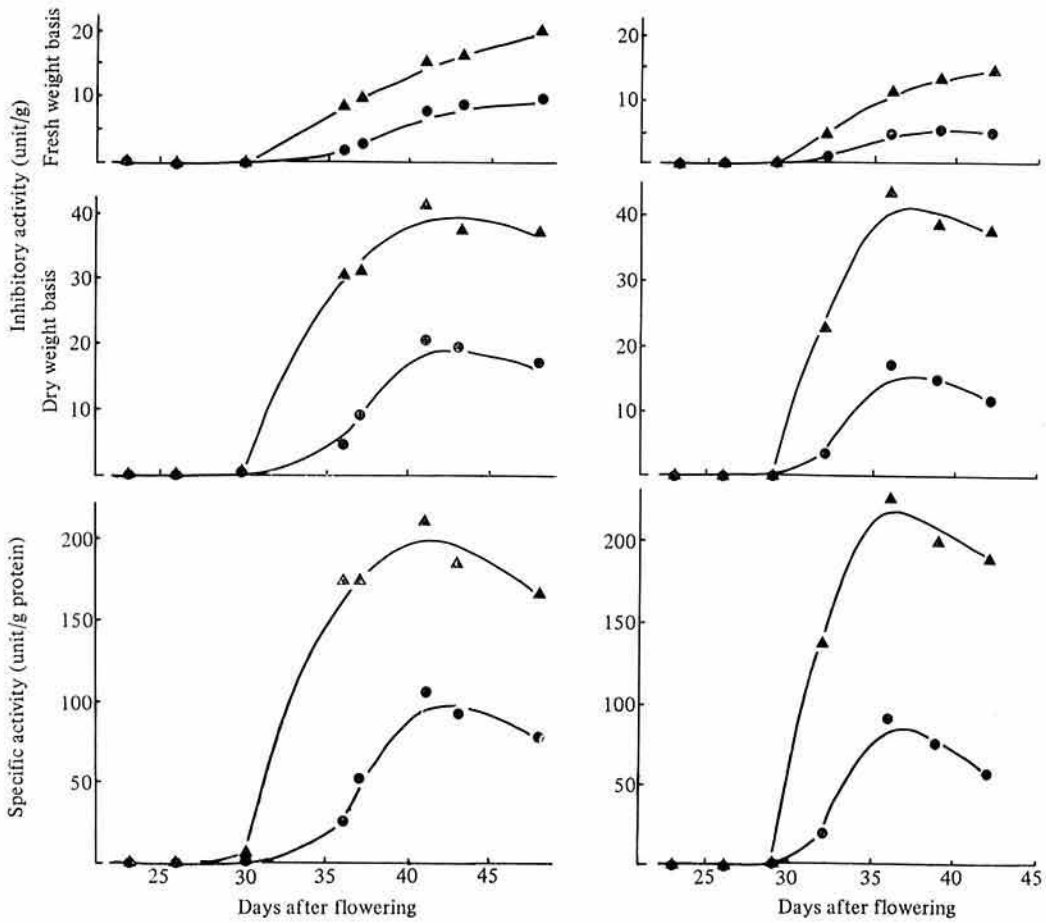

Fig. 1. Development of activity of trypsin inhibitors (●) and that of chymotrypsin inhibitors (▲) in the course of seed development of two winged bean varieties, UPS-31 (left) and Tpt-2 (right).

tubers of the winged bean, which functions as a storage and propagative organ similar to the seeds, also contained the protease inhibitors. This suggests that these inhibitors might play a role in protecting these organs from predators.

References

1) Claydon, A.: A review of the nutritional value of the winged bean *Psophocarpus tetragonolobus* (L.) DC. with special reference to Papua New Guinea. *Sci. New Guinea*, 3, 103–114 (1975).
2) Data, E. S. & Pratt, H. K.: Patterns of pod growth, development, and respiration in the winged bean (*Psophocarpus tetragonolobus*). *Trop. Agric.*, 57, 309–317 (1980).
3) de Lumen, B. O. & Salamat, L. A.: Trypsin inhibitor activity in winged bean (*Psophocarpus tetragonolobus*) and the possible role of tannin. *J. Agr. Food Chem.*, 28, 533–536 (1980).
4) del Rosalio, R. R. et al.: The trypsin inhibitor activity of legume seeds. *Phil. Agr.*, 63, 339–344 (1980).
5) Lowry, O. H. et al.: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193, 265–275 (1951).
6) Masefield, G. B.: *Psophocarpus tetragonolobus*, a crop with a future? *Field Crop Abs.*, 26, 157–160 (1973).
7) Schwert, G. W. & Takenaka, T.: A spectrophotometric determination of trypsin and chymotrypsin. *Biochem. Biophys. Acta*, 16, 570–575 (1955).

(Received for publication, August 16, 1983)