Appearance of Lectin in Winged Bean Pods during Seed Development after Flowering

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Fluctuation of size and formation of lectins in winged bean pods at different growth stages after flowering were examined. Fresh weight of the shell increased remarkably until day 30 and decreased gradually thereafter. In contrast, the seeds were growing actively from day 30 and reached maturity about 65 days after flowering. After 30 days, both the shell and the seeds agglutinated human erythrocytes but not rabbit erythrocytes. By using rabbit erythrocytes, the hemagglutinating activity was first found after 40 days, both in the shell and in the seed. Since basic and acidic lectins found in the mature seeds differed from each other in blood group specific agglutination, these results suggested that the acidic lectin appeared after 30 days and the basic lectin only after 40 days. Antigenicity against the basic lectin was not detected until day 40, thus revealing that the basic lectin appeared later than the acidic lectin.

Winged bean (*Psophocarpus tetragonolobus* (L.) DC), indigenous to the humid tropics, has been widely cultivated in Southeast Asia and other tropical areas for centuries (Pospisil et al., 1971). All parts of this plant are said to be edible, i.e., seeds, green pods, flowers, shoots, and tubers. The mature seeds, having been brought to our attention as constituting a promising legume in the tropics, have been reported to have favorable qualities as one of the food resources (Cerny et al., 1971; Pospisil et al., 1971; Higuchi et al., 1982). At the same time, however, the seeds are very toxic in their raw state, causing the death of rats within a short period (Jaffé and Korte, 1976).

Occurrence and some properties of antinutritional factors such as protease inhibitors and lectin in mature seeds of winged bean have been reported (Kortt, 1979; Pueppke, 1979). In our previous studies, two electrophoretically different lectins, basic and acidic lectins, were found in the mature seeds, and each of these two lectins was isolated by affinity chromatography (Higuchi and Iwai, 1985; Higuchi et al., 1986). Furthermore, we have indicated that the lectins are main factors in the deleterious and lethal effect of raw winged bean seeds on rats and that the toxicities of the lectins are initiated by their binding action to the intestinal epithelial cells (Higuchi et al., 1983, 1984).

Lectin activity is known to be destroyed with 30 min of autoclaving at 110 °C and 1.1 kg/cm (Liener, 1974). However, the activity of lectin in bean meal has been found extremely resistant to a dry-heat treatment as strong as at 110 °C (de Muelenaere, 1964). The heat treatment used in the daily cooking varies place to place, and it may be able to inactivate lectin activity only partially.

In view of the fact that the most popular edible part of winged bean is the young pod, shells and seeds, it is necessary to evaluate the lectin activity during the premature periods of winged bean. However, no systematic study has been done on the lectin in young winged bean. In the present study we attempted to investigate the appearance of lectin in winged bean during seed development after flowering.

MATERIALS AND METHODS

Materials. Winged bean seeds, cultivar grown in Sri Lanka (Strain No. UPS-99), were planted at experimental farm of Kochi University. The pods were harvested at every 5–10 days after flowering.

Extraction of Lectin. Winged bean pods harvested at different growth stages were weighed and then divided into two portions, shells (pericarps) and seeds. After fresh weights and moisture contents were measured, the shells and the seeds were ground separately and suspended in 10 volumes of 0.9% NaCl. The suspension was gently stirred at 4 °C for 2 h and then centrifuged at 8000*g* for 20 min at 4 °C. The supernatant was examined for the hemagglutinating activity, protein, and antigenicity. Protein was measured by the method of Lowry et al. (1951), using bovine serum albumin as the standard.

Hemagglutinating Activity. Hemagglutination was tested with serial 2-fold dilution method in a microtiter plate (Gartner and Podleski, 1975). Trypsinized erythrocytes were prepared by treatment of a 4% cell suspension in phosphate-buffered saline (7 mM phosphate–0.15 M NaCl, pH 7.2) with trypsin (1 mg/mL) at 37 °C for 1 h. Each sample (25 μL) was serially diluted with phosphate-buffered saline (25 μL), a 4% suspension of trypsinized erythrocytes (10 μL) was added to each well of a microtiter plate, and agglutination was determined after incubation at 37 °C for 1 h. Hemagglutinating activity (titer) is defined as the reciprocal of the greatest dilution at which aggultination occurred.

Immunological Methods. Rabbit antiserum against the winged bean basic lectin was prepared with injection of the purified lectin (1 mg) emulsified in Freund's complete adjuvant. The basic lectin was purified from the mature seeds of winged bean by DEAE-Sephadex A-50 and affinity chromatography on (*p*-aminophenyl)-β-D-galactopyranoside-bound Sepharose 4B (Higuchi and Iwai, 1985). The blood was drawn from the ear vein of the rabbit 10 days after the booster injection, and the serum was separated by centrifugation at 3000 rpm for 10 min. The reactivity to the antiserum was determined by the double-immunodiffusion method of Ouchterlony (Garvey et al., 1977).

RESULTS AND DISCUSSION

Winged Bean at Different Growth Stages. Figure 1 shows the winged bean at different growth stages, used in the present study. The pods, yellowish green in their young stages, turn brownish and after 60 days become very brown as they start drying.

Changes in Length and Weight of the Pods after Flowering. Fresh weight of the whole pod increased remarkably until day 30 and decreased gradually thereafter, as given in Table I. The decrease in fresh weight after 40 days reflects the loss of moisture content. The average

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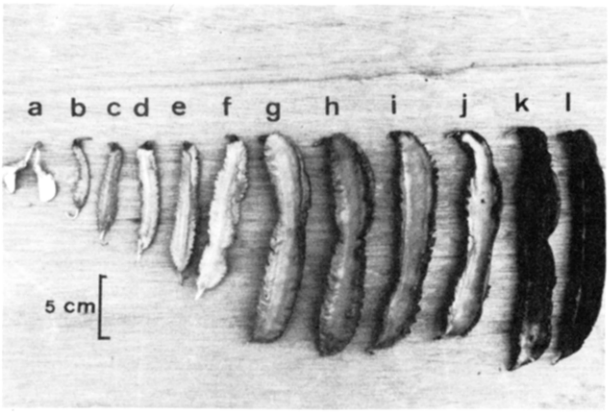

Figure 1. Winged bean at different growth stages after flowering: a, flower; b, 7th day; c, 10th day; d, 15th day; e, 20th day; f, 25th day; g, 30th day; h, 40th day; i, 50th day; j, 55th day; k, 60th day; l, 65th day.

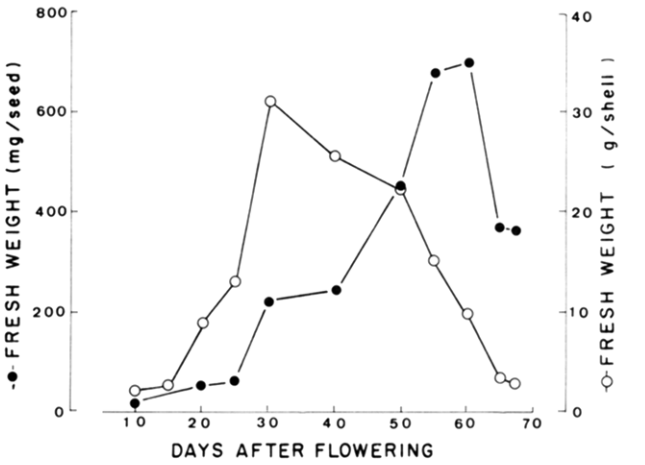

Figure 2. Changes in fresh weight of shell and seed of winged bean during seed development after flowering. Fresh weight of individual shell (○) is expressed in grams, and fresh weight of one seed (●), in milligrams.

Table I. Changes in Length and Weight of Pod after Flowering

days after flowering	length,[a] cm	weight,[a] g fresh	weight,[a] g dry
7	3.6	0.3	
10	6.4	1.6	0.1
15	9.2	3.1	0.2
20	14.4	9.1	0.7
25	17.0	16.5	2.9
30	18.8	32.1	4.6
40	18.5	27.1	4.9
50	14.5	25.7	5.1
55	14.0	20.7	5.6
60	18.0	15.4	5.7

[a] Average of two to five samples. [b] Dry weight calculated by correcting for moisture content.

length of the pods during days 50–55 (about 14 cm) was shorter than that during days 30–40 (about 19 cm). This result suggests that the number of days after flowering is more indicative of growth stages than the length of the pods. The pattern change in the shell weight (Figure 2) was similar to that of the whole pods. In contrast, the seeds were growing actively from day 30 and reached maximum weight in 60 days. The pods dried almost completely after days 65–67, thus suggesting that the winged bean had reached maturity.

Hemagglutinating Activity during Seed Development. The hemagglutinating activity at different growth stages of winged bean is shown in Table II. When human type A and O erythrocytes were used, the hemagglutinating activity was first detected after 30 days, both in the shell and in the seed, and then rose rapidly until day 65 after flowering, remaining constant thereafter. With the rabbit erythrocytes, however, such activity was first found after 40 days. At the day 30, the activity was detected neither in the shell nor in the seed. Having no lectin activity, the immature pods harvested 10–20 days after flowering, commonly consumed as foods in the cultivating areas, were thus assured to be nontoxic. Unlike our result, lectin activity was found in green shells but not in seeds at an early stage of pod development, although the exact growth stage of the winged bean was not shown (Yagi et al., 1985).

Our previous studies demonstrated that the basic and acidic lectins in the mature seeds differed from each other in blood group specific agglutination; the basic lectin agglutinated human types A and B and rabbit erythrocytes but not type O erythrocytes, while the acidic lectin strongly agglutinated type O in addition to types A and B but not rabbit erythrocytes (Higuchi and Iwai, 1985; Higuchi et al., 1986). As indicated above, the shell and the seed after 30 days already showed the activity with type O but not with rabbit erythrocytes. These results suggest that the acidic lectin appeared after 30 days and the basic lectin only after 40 days.

The extracts from the seeds after 40 days agglutinated human type O as strong as type A, while they agglutinated rabbit erythrocytes significantly less (Table II). These patterns remained unchanged during the maturation progressed. A similar result was obtained with respect to the shell. This is inconsistent with the recent report by Yagi et al. (1985) that the hemagglutination behavior of immature shells of winged bean was different from those of seeds. However, they agree with us in that the hemagglutination pattern of the seeds did not change during maturation.

Table II. Hemagglutinating Activity and Antigenicity of Winged Bean during Seed Development

days after flowering	sp act.,[a] titer/mg protein: shell type A	shell type O	shell rabbit	seed type A	seed type O	seed rabbit	antigenicity[b]: shell	seed
10	–	–	–	–	–	–	–	–
20	–	–	–	–	–	–	–	–
30	12.5	8.76	–	30.4	24.8	–	–	–
40	36.5	28.6	7.82	88.3	60.7	11.2	+	+
50	115.3	114.4	28.8	543	504	68.0	+	+
60	342	319	85.6	1133	1039	142.4	+	+
65	683	635	189.6	1851	1833	230	+	+
67	708	661	190.2	1872	1844	234	+	+

[a] Hemagglutinating activity determined by the serial 2-fold dilution method using a 4% suspension of trypsinized human types A and O and rabbit erythrocytes. [b] Antigenicity determined by a double-immunodiffusion test using rabbit antiserum against the winged bean basic lectin.

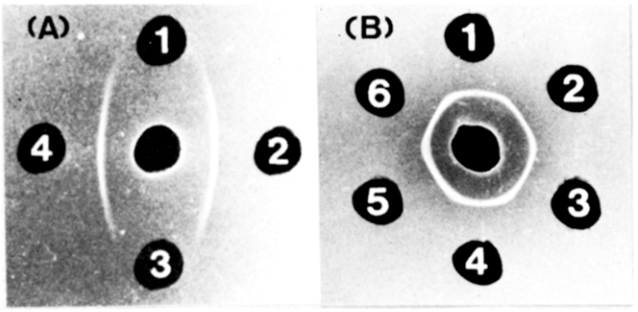

Figure 3. Immunodiffusion pattern of the extracts from winged bean. Double-immunodiffusion test was carried out in 1.5% agarose gel in 25 mM phosphate buffer (pH 7.2), which allowed diffusion overnight at 37 °C. Antiserum raised against the purified basic lectin was placed in the center well. (A) The seed extract after 30 days was placed in wells 1 and 3 and the seed extract after 40 days in wells 2 and 4. (B) The seed extract after 65 days was placed in wells 1 and 4, the shell extract after 65 days in wells 2 and 5, and extract from mature seeds of Thailand cultivar in wells 3 and 6.

Antigenicity during Seed Development. When the shells and the seeds were examined by the double-immunodiffusion test with the antiserum raised against the purified winged bean basic lectin, the extracts from seed after 40 days gave a single precipitin line with the antiserum, but the extracts after 30 days gave none (Figure 3A). As previously reported, the winged bean acidic lectin did not cross-react with the antiserum against the basic lectin, indicating an immunological difference of these lectins (Higuchi et al., 1986). No existence of the antigenicity against the basic lectin after 30 days suggested, therefore, that the basic lectin was not present either in the shell or in the seed after 30 days and agreed with the results obtained by determination of the hemagglutinating activity. These results clearly indicated that the basic lectin appeared later than the acidic lectin. Antigenicity of the shells and the seeds is summarized in Table II.

The antiserum used in the present study was raised against the winged bean basic lectin purified from the Thailand cultivar. The extracts from the Sri Lanka cultivar of winged bean gave a single precipitin line with the antiserum and completely fused with a line obtained by the winged bean basic lectin purified from the mature seeds of the Thailand cultivar (Figure 3B). The winged bean obtained from Indonesia also reacted with the antiserm. These results suggested that the antigenic determinants on the winged bean basic lectin did not vary by cultivar. Pusztai et al. (1981) reported that antigenic determinants of *Phaseolus vulgaris* lectin were not common among the cultivars. We have suggested that a tryptophan residue essential to the sugar-binding site of the winged bean basic lectin does not contribute to the antigenic site (Higuchi et al., 1985). Further immunological properties of the two lectins in winged bean have not yet been established. We now contemplate preparing antiserum against the acidic lectin in order to better compare the antigenicities of the two lectins in winged bean.

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