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Phytohaemagglutinins in the Winged Bean *Psophocarpus tetragonolobus* L. DC.

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Abstract : Seeds from eleven cultivars of *Psophocarpus tetragonolobus L.* were screened for phytohaemagglutinin activity. The levels ranged from 3,200 to 25,600 haemagglutinating units/g sample, on a fresh weight basis. Tubers and leaves of two indigenous cultivars tested, also showed the presence of phytohaemagglutinins but at a lower profile in comparison to that of seeds. Soaking the seeds for a ten-hour period does not seem to have any influence in decreasing the levels of this anti-nutritional factor. But phytohaemagglutinins present in the winged bean seeds were found to be thermolabile.

1. Introduction

The importance of the winged bean as a potential source of protein had been recognised since 1975.[14] However one of the principal drawbacks in the utilisation of winged bean protein is the presence of anti-nutritional factors typical of many legumes. Of these, phytohaemagglutinins (PHA) have not received much attention so far.

Non-specific haemagglutinating activity of the winged bean seed extract was first noticed by Renkonen[12] and further substantiated by Schertz *et al.*[13] Bhatia and Allen[2] found that winged bean agglutinating extract did not react strongly with the rare 'Bombay' group which are H-antigen deficient. Data with regard to the quantification of PHA in the edible portions of winged bean is nil.[1,11] Therefore, an attempt was made in this study to assay the levels of PHA in a range of cultivars of winged bean grown in Sri Lanka.

2. Experimental

2.1 Materials :

Winged bean samples : Seeds belonging to eleven cultivars of winged bean were studied for PHA activity. Of these, 4 are indigenous cultivars of Sri Lanka, namely Sri Lanka Selections (SLS) - SLS'_1, SLS'_6, SLS_7, and SLS_{20}. Of the 7 introduced cultivars,

(i) Six are from Papua New Guinea (University of Papua New Guinea Selections (UPS) – UPS_{31}, UPS_{45}, UPS_{46}, UPS_{59} UPS_{66}, and Chimbu.

(ii) One is from Indonesia: LBNC - 1.

In addition, leaves and tubers of two cultivars from Sri Lanka (SLS_1 and SLS_7) were also investigated.

Erythrocytes: The blood was collected in anti-coagulant, ethylene diamine tetra-acetate sodium (EDTA-Na; 2.0mg/10ml) from healthy student volunteers through vein-puncture. The haemoglobin concentrations were estimated within 3 hours, and erythrocytes were prepared from the blood of those donors, who had haemoglobin concentration within the normal range of 13.0 - 18.0 g/dl.[3]

Trypsin (1.0%) solution : 50mg of trypsin (BDH Chemicals, England; activity :- 3.0ml of 0.004 percent trypsin hydrolyses 10mg caesin in 5ml in one hour at 40°C) was dissolved in 5.0ml of 0.05N HCI and the solution stored at 4°C. A 0.1 % working solution was made by diluting 1 part of stock solution with 9 parts of buffered saline.[3]

Saline: 0.89% NaCl solution.

Sample preparation : One gram sample from raw mature seed and tuber of each of the winged bean cultivar was extracted with 50ml phosphate buffered saline (PBS), 0.1M, pH 7.6, by homogenising in a Waring blendor for 5 minutes and clarified by centrifugation. 0.5ml of the resulting extract was used for the assay. Mature leaves were dried at 60°C for approximately 6 hours until the moisture content decreased within a range of 5-10 percent, and one gram sample was homogenised in the same pattern as that of seeds.

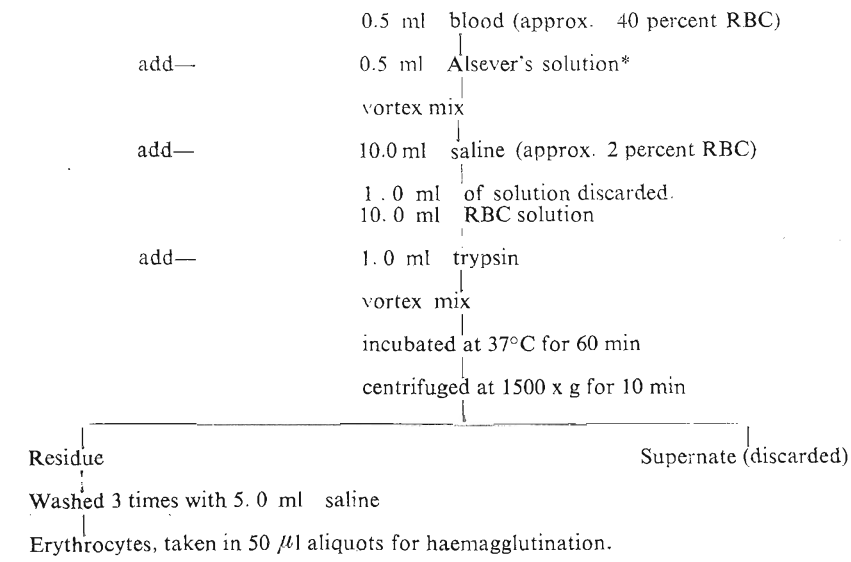
3. Method

Haemagglutination activity was studied in the winged bean extracts with saline -washed human erythrocytes (O-group) by a modification of the serial dilution technique of Liener and Hill.[10] The modified procedure was as follows :--

(i) Human erythrocytes were trypsinated according to Liener,[7] instead of being papain-treated for better agglutination. Trypsin concentration was reduced from 1.0 percent level to 0.1percent to avoid haemolysis of the cells during trypsination.[3]

(ii) Clumping of the erythrocytes was carried out in microtitre plates instead of test tubes. This facilitated easy recognition by the naked eye.

Schematic outline for the preparation of erythrocytes for haemagglutination is shown in Fig. 1. The blood (0.5ml) was added to an equal volume (0.5ml) of Alsever's solution and mixed with 10.0ml saline to obtain a final red blood cell concentration of approximately 2 percent. From this 11.0ml of erythrocyte suspension, 1.0ml was discarded and to the remaining 10.0ml of 2 percent erythrocyte suspension, 1.0ml of 0.1 percent trypsin solution was added and incubation was carried out at 37°C for one hour. The trypsinated cells were separated by centrifugation at 1500 × g or 10 minutes and washed three times with 5.0ml saline. These erythrocytes were used in haemagglutination.



**Alsever's solution* : consisted of glucose (20.5g), sodium citrate (8.0g), citric acid (0.55g), sodium chloride (4.2g) ; and made upto one litre at pH 6.1.

Figure 1. Schematic outline for the preparation of erythrocytes for haemagglutination.

In performing the haemagglutination tests, 0.5ml (500μl) of each winged bean extract was diluted in twofold increments (1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256 and 1:512) to a final dilution of 1:512 in 0.5ml saline in a micro-titre plate. 50μl of a suspension of trypsinated erythrocytes were added to each well and incubated at room temperature (28 $\pm$ 2°C) for 5 hours. Agglutination was read visually by noting the clumping of the erythrocytes.

A series of wells containing only 0.5ml of saline plus 50μl of the erythrocytes served as a negative control for this purpose. The degree of agglutination was graded as positive (+ve) or negative (—ve). One haemagglutination unit (HU) is defined as the least amount of haemagglutination occurring under the conditions specified herein: the haemagglutinating activity of the winged bean extract was calculated as given by Liener and Hill :[10]

$$\text{HU/g sample} = \frac{D_a \times D_b \times S}{v}$$

$$= \frac{D_a \times D_b \times 50}{0.5}$$

$$= 100 \times D_a \times D_b$$

where, v = volume of extract in well 1 (= 0.5ml)

D_a = dilution factor in extract in well 1 (=1 unless original extract was diluted).

D_b = dilution factor of well containing positive evidence for haemagglutination.

S = ml original extract/g winged bean extract (=50).

4. Results and Discussion

Since it has been reported[15] that seeds of the *Psophocarpus tetragonolobus* contain highest concentrations of PHAs, in this study eleven of the winged bean cultivars with high protein content (above 36.1 percent) were screened for PHA activity.

From table 1, it is observed that haemagglutination titre (reciprocal) ranged from 32 (in UPS_{31}) to 256 (in SLS_1 and UPS_{46}) with seven of the cultivars (SLS_7, SLS_6, SLS_{20}, UPS_{66}, Chimbu and LBNC-1 exhibiting a modal value of 128. Haemagglutinating activity measured in haemagglutinating units (HU)/g sample, ranged from 3,200 to 25,600. The data obtained reveals that the modal value lies at 12,800 HU/g sample. An eight-fold variation in haemagglutinating activity is seen among the cultivars. This finding is comparable with that of soybean haemagglutinating activity where a sevenfold variation among the varieties have been reported by Kakade *et al.*[6]

TABLE 1. Phytohaemagglutinin activity of raw seed extract* exhibited in trypsinated human erythrocytes

Cultivar*†	Country of origin	Haemagglutination titre (reciprocal)	HU/g sample**
SLS_1	Sri Lanka	256	25,600
SLS_6	Sri Lanka	128	12,800
SLS_7	Sri Lanka	128	12,800
SLS_{20}	Sri Lanka	128	12,800
UPS_{31}	Papua New Guinea	32	3,200
UPS_{45}	Papua New Guinea	64	6,400
UPS_{46}	Papua New Guinea	256	25,600
UPS_{59}	Papua New Guinea	128	12,800
UPS_{66}	Papua New Guinea	128	12,800
Chimbu	Papua New Guinea	128	12,800
LBNC-1	Indonesia	128	12,800

* Extracted by homogenising one gram sample in 50 ml phosphate buffered saline, 0.1M, pH 7.6, in a Waring blendor for 5 minutes and clarified by centrifugation.

** Expressed as haemagglutinating units (HU) per g sample, as defined by Liener and Hill (1953).

*† SLS – Sri Lanka Selections; UPS – University of Papua New Guinea Selections.

Phytohaemagglutinin activity present in the tubers and leaves of two indigenous cultivars (SLS_1 and SLS_7) are shown in Table 2. The haemagglutinin activity in the dried leaves and tubers are 800 and 100 HU/g sample respectively. The lower profile of PHA activity in the tubers, in comparison to that of seeds, may be correlated with the lower protein content of these edible portions. It is appropriate to note that the protein contents of the seeds and raw tubers analysed in this study had been estimated and are in the range of 29.8 to 42.5 g/100g and 2.7 to 7.1 g/100g respectively on a fresh weight basis.

TABLE 2. Phytohaemagglutinin activity of raw tuber and dried leaf extracts*

Cultivar	Haemagglutination titre (reciprocal)	HU/g sample** on fresh weight basis
Tuber :		
SLS_1	1	100
SLS_7	1	100
Leaf :		
SLS_1	8	800
SLS_7	8	800

* One gram sample was extracted with 50ml phosphate buffered saline (PBS), 0. 1M, pH 7.6, by homogenising in a Waring blendor for 5 minutes followed by centrifugation for clarification.

** Expressed as haemagglutinating units (HU)/g sample as defined by Liener & Hill (1953).

The data relating to the inactivation of PHA of seeds with increasing time of heating from 5 to 45 minutes is provided in Table 3. Soaking the seeds for 10 hours in tap water did not show any positive influence in decreasing the levels of PHA. But it is evident from the observations that haemagglutinating activity within the first five minutes of heating reached a lower level of 800 HU/g sample in both the treatments. These observations reveal the thermol abile nature of PHA. Further decrease in the haemagglutinating activity was relatively slow with increasing time up to 45 minutes. The decreasing trend of the PHA activity from 5 to 45 minutes follows more or less a similar pattern in the soaked as well as unsoaked winged been seeds.

TABLE 3. Effect of varying time of moist heat treatment of phytohaemagglutinins

Conditioning treatments	Haemagglutinating activity in SLS_1 seeds (HU/g sample) on fresh weight basis)*							
	Duration of heating time (min)							
	0	5	10	15	20	25	30	45
1. unsoaked, boiled in water†	25,600	800	800	800	800	400	200	200
2. soaked and boiled in water‡	25,600	800	400	200	200	200	200	200

* Haemagglutinating activity of SLS_1 raw mature seed is 25,600 HU/g sample on a fresh weight basis. This value remained constant over a period of 10 hours soaking (Mean of four determinations.)

† Hundred unsoaked seeds were boiled in about three volumes of tap water for 5, 10, 15, 20, 25, 30 and 45 minutes.

‡ Hundred seeds were washed and soaked overnight in about three volumes of tap water and boiled for 5, 10, 15, 20, 25, 30 and 45 minutes.

The biological significance of the PHA is manifold, according to Toms and Westtern.[16] These include toxicity, inactivation of tumour cells, a therapeutic effect on aplastic anaemia, protection of lymphocytes against nitrogen-mustard and depression of the immune response in rodents. However, there is divided opinion

among scientists regarding the toxic nature of PHA. Liener[7] reported growth tetardation in growing rats, when raw diets containing soybean were fed. Further studies by Liener[9] showed that soybean PHA is readily inactivated by pepsin; thus it may be inferred that it is probably inactivated during its passage through the stomach. It had also been pointed out that, undigested PHA would have to be absorbed from the intestine to come into contact with erythrocytes, an occurrence which seems unlikely because of the high molecular weight of the PHA. On the contrary, Jaffe[5] had observed that the toxic haemagglutinin of the seeds of *Phaseolus vulgaris* is not always completely destroyed and this was proved by the agglutination test as well as feeding trials in humans which resulted in diarrhoea and other signs of toxicity.

Feeding trials on rats may provide us with the information whether the winged bean PHA exerts any toxic effect when taken in the raw state.

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