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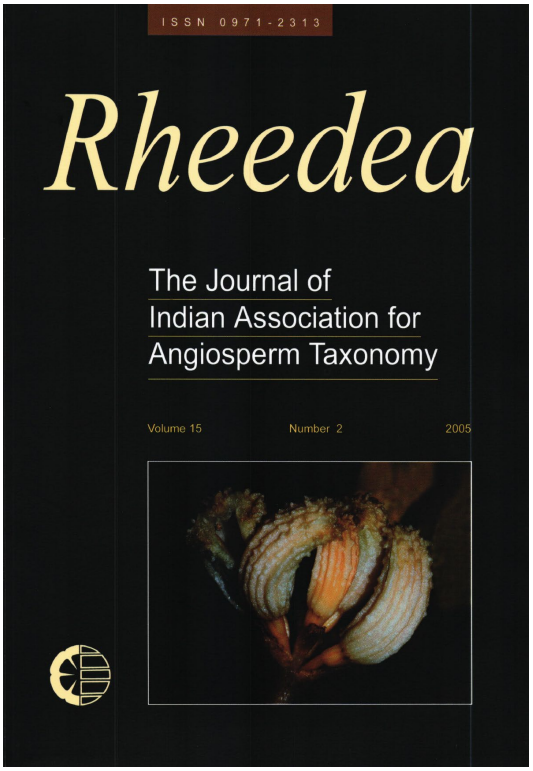
Mamatha Rao & B.U. Shalini



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Lectins in Seeds of Sixteen Species of Edible Legumes: Distribution and Infraspecific Variation

Mamatha Rao and B.U. Shalini

Department of Botany, Bangalore University, Bangalore 560 056, Karnataka, India.
E-mail : krao@vsnl.com

Abstract

Distribution of lectins in seeds of 87 cultivars belonging to 16 edible species in 12 genera of Papilionoideae was studied. Variations in the strength of agglutination, titre value, blood group as well as sugar specificity and preferential agglutination of erythrocytes of a particular blood group were observed at infraspecific level. Specificity of the cultivars KM-61 and TTB-7 1995 of *Cajanus cajan* to the human B (and AB) group of erythrocytes is a significant observation.

Keywords: Legumes, Lectins, Infraspecific variations, Blood group specificity

Introduction

Lectins are naturally occurring carbohydrate binding proteins of non-immune origin. Lectins agglutinate cells and/or precipitate carbohydrates or glycoconjugates. This makes the study of lectins in edible legumes compelling, particularly in terms of their application in human health and nutrition, besides in biochemical and biomedical research. Con A, the seed lectin of *Canavalia ensiformis*, is one of the most widely used research tool in cell recognition and as a molecular probe. The discovery that the seed lectin of *Phaseolus vulgaris* (PHA) induces mitoses in normal human leucocytes (Nowell, 1960) has immensely helped studying mammalian karyotypes with great ease. Some legume lectins were used in cancer diagnostics: *Arachis hypogaea* in colon cancer, *Bauhinia purpurea* in lung and thyroid cancer, *Macrotyloma uniflorum in* lung cancer, *Glycine max* in cervical cancer and *Ulex europeus* in prostate and endometrial cancer (Hall *et al.*, 1998). Some legume seed lectins are useful in determining human and animal blood groups without recourse to antisera (Kameswara Rao, 2000). There were no antisera to distinguish human blood subgroup A1 from subgroup A2. It has been found that seed lectin of *Macrotyloma uniflorum* can be used for this purpose (Sathyananda, 1989).

Many have studied plant lectins (Toms & Western, 1971; Toms, 1981; Sandhu & Reen, 1982; Sathyananda, 1989; Sathyanarayan Bhat, 1993; Sangeetaa, 1994; Sharon, 1994; Shubharani, 1995) though studies on lectins in edible legumes are rather scanty. Sharu Raj (1990) has brought out infraspecific variation in qualitative and quantitative distribution of lectins among the cultivars of rice and tomato. This paper presents qualitative and quantitative infraspecifc variation in lectin distribution of edible legume seeds.

Methodology

Seeds of 87 cultivars belonging to 16 species in 12 genera of Papilionoideae (Fabaceae) were obtained from (a) Indian Agricultural Research Institute, New Delhi, (b) Indian Institute of Horticultural Research, (c) University of Agricultural Science, (d) Indian Agro Seeds (e) Indo-American Hybrid seeds (all in Bangalore) (f) National Institute of Agricultural Botany, Cambridge, UK and (g) other commercial outlets in Bangalore and screened for lectins (Table 1). Lectin activity was assayed through the agglutination of erythrocytes (erythrocyte agglutination, erythroagglutination, haemagglutination).

One gram of seed was finely ground in a glass mortar with a pestle and was suspended overnight in 10 ml of petroleum ether to remove lipids. The suspension was centrifuged at 10,000 rpm for 5 min. The pellet was dried to evaporate residual ether and was suspended in 10 ml of 0.02 M phosphate buffered

saline (PBS) at pH 7.2, containing 0.15 M NaCl and kept overnight at 4^{0}C. The suspension was centrifuged at 10,000 rpm for 10 min. The supernatant was collected and tested immediately or stored in a refrigerator till used.

Phenolic interference was removed from PBS extracts using polyvinyl pyrrolidone (PVP; 10% W\V) before testing for erythrocyte agglutination (Toms & Western, 1971). Lipid interference was removed using petroleum ether. A number of secondary metabolites, including saponins which lyse human erythrocytes, were removed by dialysis against PBS for ten to twelve hours, using Spectropor dialysis tubing, after precipitating proteins in PBS saturated with ammonium sulphate (Satyananda, 1989). The samples were tested after dialysis for the presence of lectins.

Samples of human blood typed in the ABO system were obtained from Blood Banks of (a) Jayadeva Institute of Cardiology, (b) Victoria Hospital and (c) K.C. General Hospital in Bangalore.

Erythrocytes belonging to A, B, AB and O human blood groups were repeatedly washed in PBS and a two per cent cell suspension of erythrocytes in PBS was used to assay agglutination activity at room temperature (Moore, 1980).

Haemagglutination activity was studied in glass VDRL plates. Fifty ml of the suspension of prepared erythrocytes were added to the wells containing 50 ml of the plant extract in PBS, both with and without PVP. The contents of the wells were mixed thoroughly using wooden toothpicks. The wells were examined after one and two hours, under 10x objective of a compound microscope to visually determine the degree of erythrocyte agglutination. The degree of agglutination was assessed and recorded as weak (+), strong (++) and very strong (+++) and total absence of agglutination was recorded as (-)(Table 1).

Five replicates were studied in all cases and tests were repeated with different samples of human blood of the same group to check for variations and errors in assessment of the strength of agglutination. Wells containing only PBS and erythrocytes or only PVP and erythrocytes served as controls.

The serial dilution technique devised for immunological assay (Kabat and Mayer, 1961) and adopted for lectin assay by Moore (1980) and Satyananda (1989) was employed to determine the lowest strength of the plant extract required for inducing visible agglutination of erythrocytes.

Six sugars were used to determine the sugar specificity of lectins. Lectins with at least a strong haemagglutination activity (++) were selected for sugar inhibition study, adopting the following procedure:

Fifty ml of seed extract were added to a well containing 50 ml of a particular sugar (200 mm) and stirred. Fifty ml of two per cent RBC suspension were added to the well. Agglutination was determined after two hours. Agglutination was inhibited if the sugar is specific for the lectin unlike in the control which contained only lectin and erythrocytes.

Lectin assay was standardized following the procedures suggested by Moore (1980), and refined by Sathyananda (1989) and Kameswara Rao and Sangeetaa (1996). The precautionary measures suggested by Kameswara Rao and Sangeetaa (1996) were followed during lectin assay.

Results and Discussion

Distribution of lectins

All the 87 cultivars belonging to 16 species studied agglutinated human erythrocytes indicating the presence of human blood group specific lectins in their seeds (Table 1). Seeds of these 87 cultivars were not studied for infraspecific variation in lectin distribution previously.

Thirty six cultivars showed erythrocyte agglutination only in presence of PVP. This meant that the crude extracts contained interfering substances. Fifty one cultivars were active in both PVP and PBS indicating that there were no strong factors in these extracts that interfere with agglutination. Cultivars of *Cyamopsis tetragonolobus* and *Trigonella foenum-graecum* showed positive results only after dialysis since they contained saponins as evidenced by erythrocyte lysis.

Strength of agglutination

There was considerable variation in the strength of agglutination among different cultivars of a species for different blood groups, both in PVP and PBS (Table 1).

All the cultivars of *Cajanus cajan*, *Canavalia ensiformis*, *Canavalia gladiata*, *Cicer arietinum*, *Vigna mungo*, *Vigna radiata* and eight cultivars of *Vigna unguiculata* agglutinated erythrocytes only after the addition of PVP (Table 1). In *Glycine max*, *Trigonella foenum-graecum* and *Vicia faba*, the addition of PVP enhanced the strength of agglutination, but in *Phaseolus vulgaris* it did not. These results indicate that the influence of substances interfering with erythrocyte agglutination is not uniform. PVP exerts a positive

Table 1: Distribution of lectin in different cultivars of edible legumes.

Sl. No.	Species / Cultivars	In PBS				In PVP			
		A	B	AB	O	A	B	AB	O
I	***Cajanus cajan* (L.) Millsp.** (=*Cajanus indicus* Spreng.) Red gram								
1	Asha	-	-	-	-	-	+	+	+
2	RA -4 95-96	-	-	-	-	+	+	+	+
3	KM-9 95-96	-	-	-	-	+	+	+	-
4	Hyderabad 3C 98	-	-	-	-	+	+	+	-
5	KM-61	-	-	-	-	-	++	+	-
6	BSMR-198	-	-	-	-	+	+	+	-
7	TTB-7 1995	-	-	-	-	-	+	+	-
II	***Canavalia ensiformis* (L.) DC.** Jack bean								
8	Local (White)	-	-	-	-	+	+	+	+
III	***Canavalia gladiata* (Jacq.) DC.** Sword bean								
9	Local (Red)	-	-	-	-	+	+	+	+
IV	***Cicer arietinum* L.** Chick pea								
10	Annigeri 1 1996	-	-	-	-	+	+	+	+
11	BG-373	-	-	-	-	+	+	+	+
12	BG-390 AUT-1 BG	-	-	-	-	+	++	+	+
13	GCP-103	-	-	-	-	+	++	+	+
14	Kabul	-	-	-	-	+	+	+	+
15	Local (UAS)	-	-	-	-	+	+	+	+
16	Local (IAS)	-	-	-	-	+	+	+	+
V	***Cyamopsis tetragonoloba* (L.) Taub.** (=*Cyamopsis psoraloides* DC.) Cluster bean								
17	F-1 Hybrid	L	L	L	L	L	L	L	L
	(After dialysis)	+	+	+	+	++	++	++	++
18	Gujarath U	L	L	L	L	L	L	L	L
	(After dialysis)	+	+	+	+	++	++	++	++
19	Pusa naubahar	L	L	L	L	L	L	L	L
	(After dialysis)	+	+	+	+	++	++	++	++
VI	***Glycine max* (L.) Merr.** (=*Glycine soja* Sieb. et Zucc.) Soyabean								
20	Hardee	++	+	+	+	++	+++	++	++
21	Monetta	++	+	+	+	+	+++	+++	+++
22	KHSB-2	++	+	+	+	+	++	++	++
23	KB-79	+	+	+	+	++	++	++	++

VII	***Lablab purpureus* (L.) Sweet.** (=*Dolichos lablab* L.) Hyacinth bean								
24	Arka jay	+ +	+ +	+ +	+	+ +	+ +	+ +	+ +
25	Arka ajay	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+
26	Arka vijay	+ + +	+ + +	+ + +	+ +	+ +	+ + +	+ +	+
27	Local	+ +	+ +	+	+	+	+ + +	+	+ +
VIII	***Macrotyloma uniflorum* (Lam.) Verdc.** (=*Dolichos biflorus* L.) Horse gram								
28	PHG-9	+ + +	-	+ +	-	+ + +	-	+ +	-
29	Madhu	+ + +	-	+ +	-	+ + +	-	+ +	-
30	PLKV-94	+ + +	-	+ + +	-	+ + +	-	+ + +	-
31	PLS-6026	+ + +	-	+ + +	-	+ + +	-	+	-
32	BGM-1	+ + +	-	+ + +	-	+ + +	-	+ +	-
33	IC-10562	+ + +	-	+ + +	-	+ + +	-	+ +	-
34	Var. 90	+ + +	-	+ +	-	+ + +	-	+ + +	-
35	Local	+ + +	-	+ + +	-	+ + +	-	+ + +	-
IX	***Phaseolus lunatus* L.** Lima bean								
36	IHR Sel-4	+	-	+	-	+	-	+	-
37	Local	+ +	-	+ +	-	+ +	-	+ +	-
X	***Phaseolus vulgaris* L.** French or Kidney bean								
38	Sel-909	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+ +
39	Burpee strangles	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
40	Sel 2	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
41	Selection 9	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
42	IIHR-220	+ + +	+ + +	+ + +	+ + +	+ +	+ + +	+ + +	+ + +
43	Open pollinated	+ +	+ +	+ +	+ + +	+ + +	+ + +	+ + +	+ + +
44	Arka komal	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
45	Dwarf	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+ + +
XI	***Pisum sativum* L.** Pea								
46	Bonneville	+	+	+ +	+	+	+	+	+
47	Arka ajit	+ + +	+ + +	+ +	+ +	+	+ +	+	+ + +
48	Azad p_1	+ + +	+ + +	+ +	+ +	+	+ + +	+ +	+ +
49	Arka-N-Delhi	+ +	+ + +	+	+ + +	+	+ + +	+ + +	+ +
XII	***Trigonella foenum-graecum* L.** Fenugreek								
50	Local	L	L	L	L	L	L	L	L
	(After dialysis)	+	+	+	+	++	++	++	++
XIII	***Vicia faba* L.** Broad bean								
51	EC-5063	+ +	+ + +	+ +	+	+	+ + +	+	+
52	2/021 - 01411 – Eweta	+	+	+	+	+	+ +	+ +	+
53	2/033 - 01559 - Aovissot	+	+	+ +	+	+	+ + +	+	+
54	2/021 - 01614 - Feligreen	+ +	+	+ +	+	+ + +	+ + +	+ +	+ + +

55	2/021 - 01617 - Perie Mora	+	+	+ +	+	+	+	+	+
56	2/033 - 01635 - Veguvio	+	+	+	+	+	+	+	+
57	2/021 - 01657 - The Sulten	+ +	+ +	+ +	+ +	+	+ +	+	+
58	2/021 - 01696 - Red Epicure	+	+ +	+	+	+	+ +	+	+ +
59	2/033 - 01704 - Puma SS_2	+ + +	+ + +	+ +	+	+ +	+ +	+	+
60	2/021 - 01722 - Optica	+	+	+	+	+ +	+ + +	+ +	+ +
61	2/021 - 01732 - Relon	+ +	+ +	+	+	+	+ + +	+	+
62	2/021 - 01738 - Bonny Cad	+	+	+	+	+	+ +	+ +	+
63	2/021 - 01767 - Jubilee Hysor	+ +	+ +	+	+	+	+ +	+ +	+
64	2/033 - 01774 - Cargo	+	+	+	+	+	+	+	+ +
65	2/032 - 01804 - Minica	+	+	+	+	+	+ +	+	+
66	2/033 - 01899 - Pronto	+ +	+ +	+	+	+	+ + +	+	+
XIV	***Vigna mungo* (L.) Hepper.** (=*Phaseolus mungo* L.) Black gram								
67	T-9	-	-	-	-	+	+	+	-
68	VB-4	-	-	-	-	+	+	+	-
XV	***Vigna radiata* (L.) R. Wilczek.** (=*Phaseolus radiatus* L.) Green gram								
69	KGG – 1	-	-	-	-	+	+	+	-
70	GM - 84 – 4	-	-	-	-	+	+	+	-
71	PDM - 86 - 199	-	-	-	-	+	+ +	+	-
72	SML 100 29—94	-	-	-	-	+	+ +	+	-
73	Kopargoan	-	-	-	-	+	+	-	-
74	LGG - 460	-	-	-	-	+	+	+	-
75	PDM	-	-	-	-	+	+	+	-
76	Pusa baisaki	-	-	-	-	+	+	+	-
77	9105	-	-	-	-	+	+	+	-
78	MGG – 341	-	-	-	-	+	+	+	-
XVI	***Vigna unguiculata* (L.) Walp.** (=*Vigna sinensis* Endl.) Cow pea								
79	TVX – 97	-	-	-	-	+	+	+	-
80	C - 152 1995	-	-	-	-	+	+ +	+	+
81	C - 152 Davangere	-	-	-	-	+	+	+	+
82	Arka Suman	-	-	-	-	+	+	+	-
83	TVX – 944	-	-	-	-	+	+	+	-
84	V – 585	+ + +	+ ++	+ +	+ + +	+ + +	+ + +	+ + +	+ +
85	Arka garima	-	-	-	-	+	+	+	+
86	KBC – 1	-	-	-	-	+	+	+	-
87	Long	-	-	-	-	+	+ +	+	+

Abbreviations used:

PBS : Phosphate buffered solution.
\+ : Lectin activity present.
L : Lysis; the line following this is the result of dialysis
PVP : Polyvinyl pyrrolidone
— : Lectin activity absent.
A, B, AB and O : Human blood groups

Table 2. Dilutions and titre values of seed extracts and erythroagglutination

Sl. No.	Species / Cultivars	In PBS				In PVP			
		A	B	AB	O	A	B	AB	O
I	***Glycine max***								
1	Hardee	10	9	10	9	10	11	10	10
2	Monetta	12	11	11	11	11	13	13	13
II	***Lablab purpureus***								
3	Arka jay	14	14	14	14	14	14	14	14
4	Arka ajay	13	13	13	13	13	13	13	13
5	Arka vijay	14	14	14	13	13	14	13	13
6	Local	12	12	11	11	11	12	11	11
III	***Macrotyloma uniflorum***								
7	PHG-9	15	0	13	0	15	0	13	0
8	Madhu	14	0	12	0	14	0	12	0
9	PLKV-94	13	0	13	0	13	0	13	0
10	PLS-6026	15	0	15	0	15	0	15	0
11	BGM-1	14	0	14	0	14	0	12	0
12	IC-10562	13	0	13	0	13	0	11	0
13	Var. 90	14	0	14	0	14	0	14	0
14	Local	14	0	14	0	14	0	14	0
IV	***Phaseolus vulgaris***								
15	Sel-909	21	21	21	21	21	21	21	21
16	Burpee stringles	21	21	21	21	21	21	21	21
17	Sel 2	23	23	23	23	23	23	23	23
18	Selection 9	21	21	21	21	21	21	21	21
19	IIHR-220	20	20	20	20	19	20	20	20
20	Open pollinated	21	21	21	21	22	22	22	22
21	Arka komal	22	22	22	22	22	22	20	20
V	***Pisum sativum***								
23	Arka ajit	14	14	12	12	12	12	11	14
24	Azad P_1	14	14	12	12	11	14	12	12
25	Arka-N-Delhi	11	14	11	14	14	14	14	12
VI	***Vicia faba***								
26	EC-5063	14	14	14	13	13	14	13	13
27	2/033 - 01559 - Aovissot	20	20	21	20	20	21	20	20
28	2/021 - 01614 - Feligreen	19	18	19	18	19	19	19	19
29	2/033 - 01704 - Puma SS_2	21	21	21	20	21	21	20	20
30	2/021 - 01722 - Optica	22	22	22	22	23	23	23	23
31	2/021 - 01732 - Relon	21	21	20	20	20	21	20	20
32	2/033 - 01899 - Pronto	22	22	21	21	23	23	21	21
VII	***Vigna unguiculata***								
33	V – 585	10	10	10	10	10	10	10	10

Abbreviations used:
PBS : 0.02 M Phosphate buffered saline.
PVP: Polyvinylpyrrollidone (10 %).
A, B, AB and O : Human blood groups.

Dilution and equivalent titre value:
9 = 128; 14 = 4,096; 19 = 1,31,072;
10 = 256; 15 = 8,192; 20 = 2,62,144;
11 = 512; 16 = 16,384; 21 = 5,24,288;
12 = 1,024 17 = 32,768; 22 = 10,48,576;
13 = 2,048 18 = 65,536; 23 = 20,97,152.

influence on haemagglutination but such influence varies depending on species and part of the plant used as a source of a lectin and the blood group. Sathyananda (1989) and Sharon (1994) also made similar observations. For these reasons, although the suggestion of Toms and Western (1971) to include PVP routinely in erythroagglutination tests is very useful, a total dependence upon PVP should be avoided. Dialysis of the extracts is necessary whenever there was no agglutination in the crude extracts.

Titre and lectin activity

The titre values varied from cultivar to cultivar of the same species though they were more uniform in *Phaseolus vulgaris* (Table 2). There was variation in the titre value among four blood groups and two cultivars of *Glycine max*, two cultivars of *Lablab purpureus*, five cultivars of *Macrotyloma uniflorum*, three cultivars of *Pisum sativum*, and seven cultivars of *Vicia faba* (Table 2). However, the titre value was

Table 3. Inhibition of Erythroagglutination by sugars

Sl.	TAXON	SUC	LAC	MAL	GAL	GLU	FRU
I	***Cicer arietinum***						
1	BG-390	I	I	I	I	I	I
2	GCP-103	I	I	I	I	I	I
II	***Glycine max***						
3	Hardee	+	I	+	I	+	+
4	Monetta	+	I	+	I	+	+
5	KHSB-2	+	I	+	I	+	+
6	KB-79	+	I	+	I	+	+
III	***Lablab purpureus***						
7	Arka jay	+	+	+	+	+	+
8	Arka ajay	+	I	+	I	+	+
9	Arka vijay	+	+	+	+	+	+
10	Local	+	I	+	I	+	+
IV	***Macrotyloma uniflorum***						
11	Madhu	+	+	+	+	+	+
12	Var. 90	+	+	+	+	+	+
13	PLS-6026	+	+	+	+	+	+
14	PLKV-94	+	+	+	+	+	+
15	PHG-9	+	+	+	+	+	+
16	BGM-1	+	+	+	+	+	+
17	IC-10562	+	+	+	+	+	+
18	Local	+	+	+	+	+	+
V	***Phaseolus vulgaris***						
19	Sel 909	+	+	+	+	+	+
20	Burpee stringles	+	+	+	+	+	+
21	Sel 2	+	+	+	+	+	+
22	Dwarf	+	+	+	+	+	+
23	Selection 9	+	+	+	+	+	+
24	IIHR-220	+	+	+	+	+	+
25	Open pollinated	+	+	+	+	+	+
26	Arka Komal	+	+	+	+	+	+

VI	***Pisum sativum***						
27	Bonneville	I	I	I	I	I	I
29	Azad P_1	I	I	I	I	I	I
30	Arka-N-Delhi	I	I	I	I	I	I
VII	***Vicia faba***						
31	EC-5063	+	+	+	+	+	+
32	Feligreen	+	+	+	+	+	+
33	Puma SS_2	+	+	+	+	+	+
34	Relon	I	I	I	I	I	I
35	Pronto	+	+	+	+	+	+
36	Jubilee Hysor	+	+	+	+	+	+
37	Optica	+	+	+	+	+	+
VIII	***Vigna unguiculata***						
38	V-585	+	I	+	I	+	+
39	Long	+	I	+	I	+	+
40	C-152	I	I	I	I	I	I

Abbreviation used:

+ : Haemagglutination present; **I** : Inhibition of Haemagglutination.

SUC: Sucrose; **LAC**: Lactose; **MAL**: Maltose; **GAL**: Galactose; **GLU**: Glucose and **FRU**: Fructose

uniform for all the blood groups in three cultivars of *Macrotyloma uniflorum*, two cultivars of *Lablab purpureus* and one cultivar of *Vigna unguiculata*. Erythrocytes of different blood groups thus require different titre strengths for visible agglutination. Titre values vary from species to species, cultivar-to-cultivar, sample to sample, and for different blood groups, depending on the concentration of lectin in the extracts.

A lectin can be converted from a non-specific human erythroagglutinant to a group specific human erythroagglutinant by the addition of PVP, as was observed in the present study, in the cultivar Azad P_1 of *Pisum sativum*, which strongly agglutinated human group B erythrocytes at a significantly lower concentration than required to agglutinate erythrocytes of the other blood groups in the presence of PVP (Tables 1, 2)

Inhibition of lectin activity by specific sugars

The presence of lectins in all the cultivars of *Cicer arietinum, Glycine max, Pisum sativum, Vigna unguiculata* studied and cultivars Arka ajay and Local of *Lablab purpureus* and Relon of *Vicia faba* was confirmed by sugar inhibition in the present study (Table 3).

Lectin activity of all the cultivars of *Cicer arietinum* and *Pisum sativum* and cultivar C-152 of *Vigna unguiculata* was inhibited by all the sugars used. This indicates that these samples possibly contain multiple lectins – different lectins with different physico-chemical properties and sugar specificities. Cultivars Hardee, Monetta, KHSB-2 and KB-79 of *Glycine max,* Arka ajay and Local of *Lablab purpureus* and V-585 and Long of *Vigna unguiculata* showed inhibition by galactose and lactose. This also indicates the presence of multiple lectins. Lectin activity of all the cultivars of *Macrotyloma uniflorum* and *Phaseolus vulgaris* studied and Arka ajay and Arka vijay of *Lablab purpureus* and all the cultivars of *Vicia faba* except Relon was not inhibited by any sugar used (Table 3). This indicates that a sugar other than those used here is specific to the lectins in them. Sugar inhibition studies showed infraspecific variation in lectin characteristics in *Lablab purpureus*, *Vicia faba* and *Vigna unguiculata* (Table 3).

Preferential agglutination

Lectins from 16 cultivars belonging to six species showed preferential agglutination of erythrocytes of a particular blood group. Cultivar Hardee of *Glycine max* showed a preference for group B (+++) against other groups (++) in PVP while cultivar Monetta preferred group A (++) to the other groups (+) (Table 1).

Cultivars BG-390 and GCP-103 of *Cicer arietinum* showed stronger agglutination of group B

erythrocytes. Cultivar Bonneville of *Pisum sativum* showed a preference for group AB. Cultivars of Perimora and Aovissot of *Vicia faba* indicated a preference for group AB, while cultivars Sulten and Pronto preferred group B and Cargo, group O. Cultivars C-152, 1995 and Long of *Vigna unguiculata* showed preferential agglutination for group B.

The titre value which reflects the minimum concentration required for agglutination and the concentration dependent preferential agglutination are related to each other. The factor of preferential agglutination of a particular blood group by a lectin is helpful in converting a non-specific lectin to a blood group specific lectin by manipulating the concentration. In the present study, *Cajanus cajan* KM-61, *Canavalia gladiata* BG-390 AUT-1 BG and GCP-103, *Vicia faba* 2/021-01722-Optica, 2/021-01732-Relon and 2/033-01899-Pronto, *Vigna radiata* PDM-86-199 and SML-10029-94, and *Vigna unguiculata* C-152 1995 and Long showed preferential agglutination for human group B erythrocytes, while *Phaseolus vulgaris* Arka ajit showed preferential agglutination for human group O erythrocytes (Table 1)

Blood group specificity

The seed lectins of all the cultivars of *Macrotyloma uniflorum* and of cultivars IHR- Sel-4 and Local of *Phaseolus lunatus* studied here showed specificity for human group A (and AB) erythrocytes (Table 1). Seed lectins of *Macrotyloma uniflorum* are routinely used in Blood Banks to distinguish the human A1 subgroup of human A group erythrocytes from those of A2 (Sathyananda, 1989). Sharu Raj (1990) and Shalini (2002) found that the seed lectin of *Phaseolus lunatus* was specific to the erythrocytes of the A2 subgroup of human A group. As there are no antisera to distinguish the human A subgroups A1 and A2 from each other, the seed lectins of *Macrotyloma uniflorum* and *Phaseolus vulgaris* would be very useful in Blood Banks to distinguish these two subgroups.

Lectins specific to the human blood group B are very rare and require characterized purification. Hence, cultivars KM 61 and TTB 7 of *Cajanus cajan* which showed specificity to B (and AB) group erythrocytes are promising. However, the possibility of using this lectin for the routine identification of B group of human blood should be confirmed by studies using a purified lectin. Cultivars that showed preferential agglutination or concentration dependent specificity to a particular human blood group (Tables 1, 2) can also be used to determine the respective human blood group. Human blood group determination kits make use of human blood group specificity of lectins eliminating the use of antisera (Kameswara Rao, 2000).

Blood group specificity helps detecting adulteration in seed meal. Seed meal of *Phaseolus lunatus* and *P. vulgaris* can be distinguished basing on blood group specificity (Table 1). Some cutivars of *Phaseolus lunatus* (IHR-Sel-4 and Local) and *Vicia faba* (Jubilee Hysor, Minica, Relon, Bonny cad, Perie Mora, Veguvio and Sutteri) have confusing seed morphology making the distinction of the two species from seeds difficult. The seed meal of the two species also is almost indistinguishable. As *Phaseolus lunatus* does not agglutinate human B and O group erythrocytes, it is possible to use this criterion to distinguish the seeds of the two species.

Taxonomic importance

Taxonomic relationships within the species of a tribe are reflected in the structure of their lectins (Feldman & Sears, 1981). Lectin production is considered as an advanced feature in legumes (Toms, 1981). Blood group A and B specific lectins are found in relatively advanced taxa (Toms and Western, 1971; Toms, 1981). In Mimosoideae, human blood group specific lectins are absent Kameswara Rao, 2000). The three genera with human blood group specific lectins reported in the present study belong to Papilionoideae.

The presence of blood group specific lectins, in contrast to non-specific lectins, is an indication of advanced position of the species from evolutionary point of view (Toms & Western, 1971; Toms, 1981). *Cajanus cajan*, *Macrotyloma uniflorum* and *Phaseolus lunatus*, with blood group specific lectins are thus to be considered more advanced than the other species in the respective genera and the tribe Phaseoleae (Toms and Western, 1971; Toms, 1981).

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Literature Cited

Feldman, R.J. & F.R. Sears 1998. The Wild Gene Resources of Wheat. Sci. Am. **244**: 98-109.

Hall, G.D., Patel, P.M. & A.S. Protheroe 1998. *Key topics in oncology.* BIOSIS Scientific Publishers Ltd., Oxford. Pp. 252, 253, 270, 271.

Hankins, C.N., Kindinger, J. & L.M. Shannon 1987. The Lectins of *Sophora japonica. Plant Physiol.* **83**: 825-829.

Kabat, E. A. & M.M. Mayer 1961. *Experimental immunochemistry*. Thomas, Springfield, Illinois. Pp. 114-119.

Kameswara Rao, C. 2000. *Database of Medicinal Plants*. Karnataka State Council for Science and Technology, Bangalore.

Kameswara Rao, C. & W. Sangeetaa 1996. Lectins in pollen and stigmas. In: Malik, C.P (Ed.), *Pollen and Spore Research*: *Emerging Strategies*. Today and Tomorrow's Printers and Publishers, New Delhi. Pp. 121-163.

Moore, B.P.L. 1980. *Serological and Immunological Methods*. The Canadian Red Cross Society, Toronto.

Moreira, R.D.A. & J.C. Perrone 1977. Purification and Partial Characterization of a Lectin from *Phaseolus vulgaris*. *Plant Physiol*. **59**: 783-787.

Nowell, P.C. 1960. Phytohaemagglutinin: an initiator of mitosis in cultures of normal human leukocytes. *Cancer Res.* **20**: 462-466.

Okamuro, J.K., Jofuku, K.D. & R.B. Goldberg 1986. Soybean seed lectin gene and flanking non-seed protein genes are developmentally regulated in transformed tobacco plants. *Proc. Natl. Acad. Sci.*, USA., **83**: 8240-8244.

Sangeetaa, M. 1994. *Lectins and Saponins in pollen and stigmas*. Ph.D. Thesis, Bangalore University, Bangalore, India.

Sandhu, R. S. & R.S. Reen 1982. Phytolectins - a review. In: Malik, C. P. (Ed.), *Annual Reviews of Plant Sciences*. Kalyani Publishers, New Delhi. Pp. 1-62.

Sathyananda, N. 1989. *Studies on legume lectins*. Ph.D. Thesis, Bangalore University, Bangalore, India.

Sathyanarayana Bhat 1993. *Plants with Antimicrobial Activity: Database and Lectin Distribution*. Ph.D. Thesis. Bangalore University, Bangalore, India.

Shalini, B.U. 2002. *Studies on Economically Important Legumes of India: Database and Distribution of Lectins and Saponins.* Ph.D. Thesis, Bangalore University, Bangalore, India.

Sharon, A. 1994. *Database of plants used in gastrointestinal disorders and effect of plant extracts on pathogenic enteric bacteria*. Ph.D. Thesis, Bangalore University, Bangalore, India.

Sharu Raj, M. 1990. *Studies on lectins in some food plants.* M.Phil. Dissertation, Bangalore University, Bangalore, India.

Shubharani, R. 1995. *Plants used in dental care in India*. Ph.D. Thesis, Bangalore University, Bangalore, India.

Toms, G. C. 1981. Lectins in leguminosae. In: Polhill, R. M. & P. H. Raven (Eds), *Advances in Legume Systematics.* Royal Botanic Gardens, Kew. Pp. 561-577.

Toms, G. C. & A. Western 1971. Phytohaemagglutins. In: Harborne, J. B., Boulter, D & B.L. Turner (Eds), *Chemotaxonomy of the Leguminosae*. Academic Press, London. Pp. 367-462.

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