Int.J.Curr.Res.Aca.Rev.2017; 5(5): 8-14

EXCELLENT PUBLISHERS

International Journal of Current Research and Academic Review

ISSN: 2347-3215 (Online) ⁘ Volume 5 ⁘ Number 5 (May-2017)

Journal homepage: http://www.ijcrar.com

doi: https://doi.org/10.20546/ijcrar.2017.505.002

Analysis of Wild Chickpea Seed Proteins for Lectin Composition

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Abstract

Lectins are carbohydrate-binding proteins of non-immunoglobulin nature and are ubiquitous in nature. Study was undertaken with an objective to determine the lectin concentration in 15 wild chickpea seeds, which was unknown till date. Presence of lectin was assayed using human (A, B, AB and O) and rabbit erythrocytes, Trypsinized rabbit erythrocytes gave considerable lectin activity under *in-vitro* conditions. A titre was observed in 4-12 wells giving 8-4096 hemagglutination units (HAU). The specific activity was found highest in *Cicer reticulatum* ILWC-292 having 589.3 titre/mg. The lectins was stable for about 4-6 h and did not inhibit simple sugar moieties, but with complex sugars like desialated fetuin only. The primary role of lectin is to provide defence thus enhancing crop yield. Based on this screening data, ILWC-292 and ILWC-21 accessions may be used as parent in executing future breeding programme.

Article Info

Accepted: 30 April 2017
Available Online: 20 May 2017

Keywords

Lectin, chickpea, hemagglutination.

Introduction

The proteins binding affinity for carbohydrate moiety taking part in protein-saccharide interactions, are known as lectins (Grubhoffer *et al*., 1997). Plant lectins are a heterogeneous group of carbohydrate-binding proteins which have the specificity to bind carbohydrates without causing their chemical transformations. Nowadays, proteins that can agglutinate red blood cells without known sugar specificity are referred to as agglutinins (Sharon and Lis, 1989). Lectins are widely distributed in nature among plants, animals, microorganisms and mainly present in dry seeds. In addition; its presence in other vegetative tissues like leaves, roots, and stem has been reported (Sharon and Lis, 1989; Goldstein and Hays 1978; Suseelan and Mitra, 2001). In the plant kingdom, seed of legumes have long been known to be a rich source of lectins, constituting up to 10% of their total protein (Lis and Sharon, 1986; Sharon and Lis, 2002).

Leguminous plants are known to synthesize certain antinutritional compounds which includes lectins as well. Lectins can be classified on the basis of their carbohydrate specificity viz. merolectins, holoectins, chimerolectins and superlectins. Literature perusal suggests that the wild plant species are rich with lectin composition thus display more resistance against their predators. The lectins from various plant species demonstrate important pharmacological properties like mitogenic (Singh *et al*., 2004), antiproliferative (Singh *et al*., 2004), anti-fungal (Zheng *et al*., 2007), nodulation enhancement (Larry *et al*., 1986) and HIV-1 reverse transcriptase inhibitory activities (Zheng *et al*., 2007). In

our previous report different 50 chickpea genotypes were evaluated for diversity of lectin composition (Bhagyawant *et al*., 2015). Chickpea lectin showing hemagglutination activity was isolated earlier from cultivated *Cicer arietinum L.* (Katre *et al*., 2004). A wide range of chickpea varieties/germplasms are now available showing important agronomic traits.

Chickpea (*Cicer arietinum* L.) belongs to the family Leguminoseae has 43 wild species. There exists different assortment of chickpea type viz. desi, kabuli and wild etc. Chickpea seeds are nutrient-dense foods providing rich content of protein and certain dietary minerals such as iron and phosphorus with antinutritional factors viz. phenolics, tannin, trypsin and lectin etc (Khatoon and Prakash, 2004). Thus, chickpea is considered a functional food or nutraceutical which provides a cheap source of protein and energy to the developing world and also acts as an important food to the affluent populations for alleviating major food related health problems. Looking at the importance of lectins in wild species, present study is undertaken to screen out wild species of chickpea for its lectin composition.

Materials and Methods

Seed material

Total 15 wild chickpea accessions (Table 1) belonging four different species viz. *Cicer reticulatum*, *Cicer pinnatifudum*, *Cicer judaicum* and *Cicer echinospermum* were used for analysis. The mature and dry seed material was obtained from Indian Institute of Pulses Research, Kanpur, India under MTA understanding.

Preparation of seed extract

Seed lectin was isolated using procedure as described by Gurjar *et al*. (1998). The dry matured seeds were finely ground in warring blender and defatted with hexane. Seed meal (1g), was added to 5 ml of Tris-HCl extraction buffer (20 mM Tris-HCl pH 7.2, containing 150 mM NaCl). The suspension was agitated for 12 h at 4°C in cold and filtered through muslin cloth. The filtrate was subsequently centrifuged at 10,000 rpm for 20 min at 4°C. The clear supernatant thus saved was further used for hemagglutination assay.

Preparation of trypsin treated erythrocytes

Trypsin treated erythrocytes for the hemagglutination assay were prepared by the method of Sharon and Lis (1989). Fresh rabbit erythrocytes were centrifuged at 2000 rpm for 10 minutes. The serum was removed and the erythrocytes were repeatedly washed with PBS. The RBC suspension (3%) was incubated with 0.05% (w/v) trypsin at 37ºC for 1 h. After incubation erythrocytes were repeatedly washed with PBS to remove trypsin and finally suspended in PBS at a concentration of 3% and used for the hemagglutination assay.

Hemagglutination assay

The hemagglutination activity was performed initially using normal human (A, B, AB and O) and rabbit erythrocytes followed by trypsin treated. Fresh erythrocytes were separated from plasma by centrifugation at 3000rpm for 4 minutes at 5-10°C and washed extensively with phosphate buffer saline (PBS). Finally, 3% suspension was prepared in PBS and hemagglutination tests were performed in standard microtitre plate by the two-fold serial dilution method (Liener *et al*, 1962). A 50µl aliquot of the erythrocyte suspension was mixed with 50µl of serially diluted lectin. Agglutination assay was examined visually after incubation for 1 h at room temperature. The unit of hemagglutination activity (U) termed as titre was expressed as the reciprocal of the highest dilution of the lectin that showed complete agglutination. The specific activity of the lectin is defined as the titre of hemagglutination per mg of protein.

Protein estimation

Protein content in the seed extracts was estimated by the Folin-Lowery method (1997) using BSA as a standard.

Carbohydrate Inhibition assay

Hemagglutination inhibition tests were performed as described in hemagglutination assay, except that serial dilutions of the sugar solution (25µl) were pre-incubated at room temperature with 25µl of the lectin (minimum concentration showing titer) for 15 minutes. 50µl of rabbit erythrocyte suspension was added, mixed and the plates read after one hour. The concentration of all the sugars used was 500mM and that of the glycoprotein used was 1mg/ml. The various sugars (Glucose, mannose, maltose, fructose, fucose, galactose N-acetyl D-glucosamine, Galactosamine, Mannosamine and Glucosamine etc.) employed in the inhibition studies. The assay was carried out at room temperature in a 96 well microtitre plate.

Results and Discussion

In the present study, lectin extracted from 15 wild chickpea seeds accessions were used for agglutination test. The 15 wild chickpea seeds extract tested, all showed the presence of lectin with varied concentration. Hemagglutination activity performed in 96 well microtitre plate with 3% rabbit blood showing agglutination appearances is shown in Figure 1. Human erythrocytes either of the human blood group A, B, AB and O nor rabbit erythrocytes, did not show any agglutinating activity at any of the concentrations that were tested. Only the trypsin treated rabbit erythrocytes were able to show positive agglutinating activity. The magnified view of the agglutination behaviour seen under the microscope is displayed in the Figure 2. Trypsinized RBCs showed higher haemagglutination titre than the untreated cells in most of the chickpea seed extracts. This is because of the mild trypsin digestion resulted in the exposure of more lectin binding sites. The reciprocal of dilution is calculated as titre value, which reflects lectin activity. Higher the titre value the higher is the lectin activity. On the other hand, absence of lectin marked a distinctive red button on the bottom of microtitre plate well.

Table.1 Hemagglutination activity of wild chickpea seed extract

S.N.	Name of species	Accession no.		Protein conc. (mg/ml)	Hemagglutination Titre unit	Specific activity Titre unit/mg
1	*Cicer reticulatum*	ILWC-21		4.21±0.07	2048	486.43
2		ILWC-292		6.95±0.09	4096	589.32
3		ILWC-142		5.01±0.11	1024	204.31
4		ILWC-139		4.55±0.06	1024	225.01
5		ILWC-17121		6.08±0.09	512	84.21
6		IC-95181		6.23±0.10	2048	328.73
7		ILWC-113		4.29±0.11	1024	238.62
8	*Cicer pinnatifidum*	ICC-17153		5.28±0.08	256	48.48
9		EC-526258		6.40±0.05	512	80.01
10		ICC-17155		4.29±0.09	256	59.62
11	*Cicer judaicum*	ILWC-17151		2.35±0.10	8	3.47
12		ICC-17149		3.45±0.12	512	148.45
13		ICC-17150		2.51±0.05	256	101.91
14		ILWC-148		6.25±0.15	2048	327.61
15	*Cicer echinospermum*	ILWC-241		3.25±0.06	128	39.34
		Average		4.74±0.09	1050.12	197.70
		Maximum		6.95±0.09	4096	589.32
		Minimum		2.35±0.10	8	3.47

Fig.1 Hemagglutination assay in micro titre plate

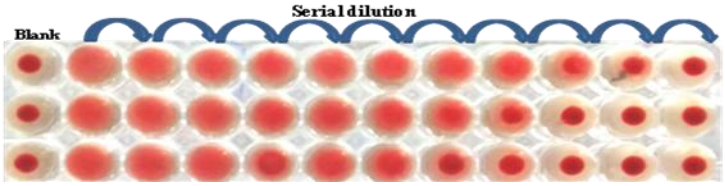


Fig.2 Magnified hemagglutination view (A) RBCs only (B) agglutinated RBCs treated with lectin

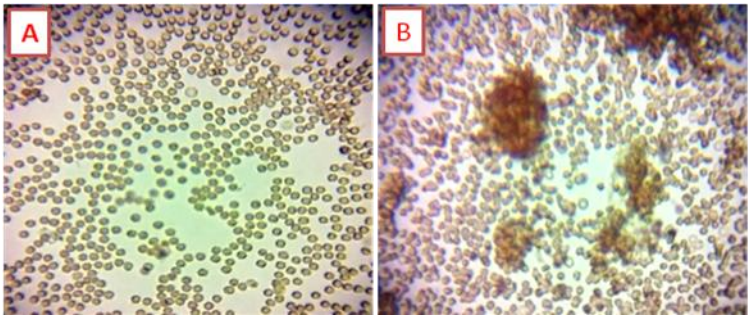


Fig.3 Distribution of protein content in seeds of chickpea

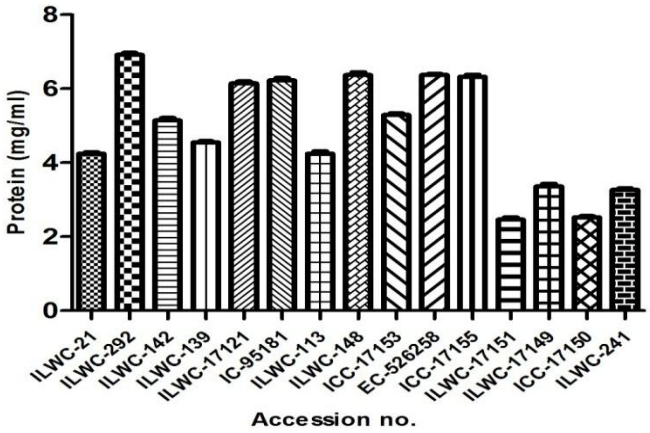
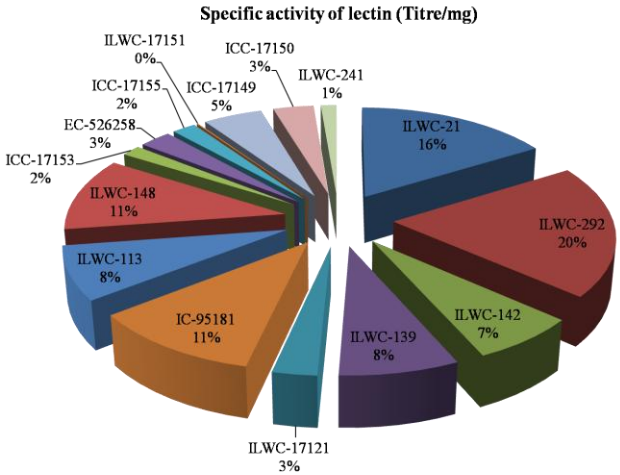


Fig.4 Specific activity of chickpea lectin



The wild accessions examined in the present study exhibited wide spectrum of protein contents. The average content of total protein across these accessions was observed to be 4.74±0.09 mg/ml. The highest amount of total protein was found in the accession ILWC-292 (6.95±0.09 mg/ml), while lowest amount was observed in ILWC-17151 (2.35±0.10 mg/ml). In chickpea seeds the average value of hemagglutination titre was 1050.12 titre unit (Figure 3), while highest hemagglutination titre value observed was 4096 unit and specific activity 589.32 titre/mg in accession ILWC-292 (Figure 4). On the other hand, the lowest activity was found in ILWC-17151 exhibiting 8 titre unit while its specific activity found was 3.47 titre/mg. Inhibition of agglutination was studied by using various sugars viz. monosaccharides, disaccharides and oligosaccharides. None of the sugars glucose, mannose, galactose their derivatives, disaccharides and disaccharides inhibited the hemagglutinating activity. Though it could inhibit a complex sugar like desialated fetuin only at a mean value of ±150µg concentration.

The presence of lectin has been detected in all number of chickpea species, maximum concentration of lectin was found in *Cicer reticulatum*, but less concentration been detected in *Cicer judecum*. The present study demonstrates that the occurrence of lectins in wild chickpea seeds is typical. Earlier studies presented the wild lectins viz. *Glycine soja* (Pueppke *et al*., 1982), *Acacia constricta* (Guzmán-Partida *et al*., 2004), *Helianthus tuberosus* L. (Suseelan *et al*., 2002), *Bradyrhizobium japonicum* (Larry and Gary, 1986), *Boletus edulis* (Zheng *et al*., 2007; Singh *et al*., 2004), *Phaseolus vulgaris* L. (Sotelo *et al*., 1994) have been reported isolated and characterised subsequently.

Lectins have been suggested as natural compounds for controlling fungi and insects which are safer than synthetic agrochemical products. In addition, they are used in structural and functional studies of complex carbohydrates as well as to analyse changes in the cell surface during physiological and pathological processes (e.g. cancer), including as main components of microarrays (Gemeiner *et al*. 2009). Antimicrobial activity of lectins has been reported in the literature against fungi and bacteria (Oliveira *et al*. 2008; Gomes *et al*. 2013). The toxicity of lectins has also been studied ensuring their safe use in human health (Rolim *et al*. 2011). The carbohydrates on cellular surfaces are distinguishable among the four different blood groups (Khan *et al*., 2008). There are reported monosaccharide determinants in the different blood groups, fucose in horse (Wu *et al*., 2009), galactose in human (Kusui K and Takasaki, 1998) and mannose in rabbit erythrocytes (Konozy *et al*., 2002). Horse, human and rabbit red blood cells may contain carbohydrate components on the cellular surface binding sites that are relatively less recognized by the *Q. fusiformis* lectin binding site. On the other hand, the carbohydrates found on the cellular surface of sheep red blood cells may contain carbohydrate units in a structure and position more specific and with higher affinity for the binding of *Q. fusiformis* lectin, subsequently increasing sheep erythrocyte agglutination. Moreover, lectin characteristics such as, multivalence may determine cross-linking interaction in binding recognition. Spatial distribution of multivalence among lectin structures may produce a higher level of specificity (Moreira *et al*., 1998; Adenike and Eretan, 2004).

In previous study, lectin from desi chickpea (*Cicer arietinum* L.) cultivar BDN 9-3 was purified and crystalized. *Cicer arietinum* L. lectin i;e CAL possessed complex-sugar specificity (Katre *et al*., 2005). The molecular weight of the native protein as determined by gel filtration using HPLC is 43 000 Da. It has been identified as a homodimer of subunit molecular weight 21 500 Da by SDS-PAGE both in the presence and in the absence of β-mercaptoethanol. The lectin was basic in nature (pI 9.0) and is a glycoprotein containing 4.5% neutral sugars. Neither human (blood groups A, B and O) nor rabbit erythrocytes are agglutinated by this lectin; however, pronase-treated versions of both have been successfully agglutinated. None of the sugars, such as glucose, mannose or galactose, nor their derivatives, disaccharides, trisaccharides and tetra saccharides has any effect on the agglutination activity of this lectin. The present analysis thus provide practical utility suggesting ILWC-292 and ILWC-21 as a parent to be used in future chickpea breeding programme

Acknowledgement

Ajay Kumar Gautam is grateful to UGC, New Delhi for providing research fellowship. Authors are also grateful to Dr. S. K. Chaturvedi, Indian Institute of Pulse Research, Kanpur, (U.P.) India for providing the seed samples. Thanks are also due to The Director, Defence Research & Development Establishment, Gwalior and Mr. P.C. Jatav for providing rabbit blood samples.

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How to cite this article:

Ajay Kumar Gautam, Nidhi Srivastava, Amit Kumar Singh Chauhan and Sameer S. Bhagyawant. 2017. Analysis of Wild Chickpea for Lectin Composition. *Int.J.Curr.Res.Aca.Rev.* 5(5), 8-14.
doi: https://doi.org/10.20546/ijcrar.2017.505.002