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Screening for Lectin Quantification in *Brassica* Spp and Vegetable Crops

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Abstract

In the present study, twenty five test entries including oilseed *Brassicas*, vegetable *Brassicas* and wild crucifers were screened for the quantification of lectins in their seeds and inflorescence. Out of twenty-five entries, nine were reported positive for the presence of lectins in their seeds while inflorescence of only one entry (*Allium cepa*) showed the presence of lectins. In seeds, the total haemagglutination activity in all the test entries ranged from 80 to 800 HU/g. The haemagglutination activity in the inflorescence of *Allium cepa* observed out to be 640 HU/ g fr.wt. The quantification data indicates variation in the haemagglutination activity among the tested entries.

Keywords: Oilseed *Brassicas*; vegetable *Brassicas*; wild crucifers; lectin; haemagglutination.

Abbreviations : PBS - Phosphate buffered saline; rpm - revolutions per minute; HU/g - Haemagglutination units per gram; HU/mg - Haemagglutination units per milligram; RBC - red blood cell

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Introduction

Lectins are a class of carbohydrate binding proteins or glycoproteins of non-immune origin capable of specific recognition of and reversible binding to, carbohydrates without altering their covalent structures (Dixon, 1981; Peumans and Van Damme, 1995) and have characteristic property of agglutinating blood cells (Van Damme et al., 1998).They are widely distributed in nature and are most abundant in plants, especially in legume seeds, where they constitute 15% of total protein. Lectins have been isolated and characterized from diverse sources; including plant seeds and roots, fungi, bacteria, algae, body fluid of invertebrates, lower invertebrates and mammalian cell membranes (Singh et al., 1999). On the basis of their overall structure, three major types of lectins are distinguished, namely merolectins, hololectins and chimerolectins (Peumans and Van Damme, 1995). Merolectins are proteins that are built exclusively of a single carbohydrate binding domain. They are small, single polypeptide proteins, which because of their monovalent nature are incapable of precipitating glycoconjugates or agglutinating cells. Examples of this group are hevein and the monomeric Man-binding proteins from orchids. Hololectins are built exclusively of carbohydrate binding domains but contains two or more such domains that are either identical or very homologous. This group comprises all lectins that have multiple binding sites and hence are capable of agglutinating cells or precipitating glycoconjugates. The majority of all known plant lectins are hololectins, because they behave as haemagglutinins. Chimerolectins are fusion proteins possessing a carbohydrate-binding domain tandemly arrayed with an unrelated domain, which has a well-defined catalytic activity (or another biological

activity) that acts independently of the carbohydrate binding domain. Depending on the number of sugar binding sites, chimerolectins behave as merolectins or hololectins. For instance, type 2 RIPs with two carbohydrate binding sites on their B chain (e.g Ricin) agglutinates cells, whereas class I plant chitinases with a single chitin binding domain do not (Peumans and Van Damme, 1995). Sequence analyses of complete plant genomes revealed that chimerolectins are very abundant in plants (Van Damme et al., 2008). Moreover, recent genome/transcriptome analyses of plants provided evidence for the occurrence of many proteins containing one or more lectin domain(s) embedded in a more or less complex multi-domain architecture (Van Damme et al., 2008).

On the basis of thorough genome/transcriptome analyses, plant lectins can be classified into twelve distinct families of evolutionary and structurally related lectin domains (Van Damme et al., 2008). These different carbohydrate-binding domains were called, in alphabetical order: (1) Agaricus bisporus agglutinin homologs, (2) amaranthins, (3) class V chitinase homologs, (4) cyanovirin family, (5) *Euonymus europaeus* agglutinin family, (6) *Galanthus nivalis* agglutinin family, (7) proteins with hevein domains, (8) jacalins, (9) proteins with a legume lectin domain, (10) LysM domains, (11) *Nicotiana tabacum* agglutinin family, and (12) ricin-B family. Each lectin domain has its own characteristic overall fold with one or more carbohydrate-binding sites.

Plant lectins perform many functions including growth regulation, carbohydrate transport and plant defense through interaction with microorganisms, insect and mammalian predators (Sharon and Lis, 2007). Plant lectins have also been shown to possess cytotoxic, fungitoxic, anti-insect and anti-nematode properties (Van Damme et al., 1998, Van Damme et al., 2007, Fitches et al., 2008, Jaber et al., 2008, Kaur et al., 2009, Shahidi-Noghabi et al., 2009, Thakur et al., 2013). Plants express a large number of highly diverse lectins, exhibiting different molecular structures and binding specificities towards endogenous (plant origin) and exogenous (non-plant origin) glycans (Van Damme et al., 2008). Thus, lectins are particularly important in the context of biological control of pests and diseases.

Brassica lectins have not been adequately studied as there are very few reports in literature. Cole (1994) reported the presence of chitin-binding lectins in wild *Brassica* species *B.fruticulosa*, *B. spinescens* in significant quantities while low levels were found in cultivated cabbage cv *Offenham compacta*. Taipalensuu et al. (1997) reported that the proteins in the family of myrosinase binding proteins, present in seeds of *Brassica napus* possess lectin activity which bind to p-aminophenyl *α*- D-mannopyranoside-agarose and N-acetylglucosamine-agarose. Lectins from *Brassica* spp./wild crucifers and vegetable *Brassicas* need extensive exploration so as to understand the variability, if exists, in terms of lectin quantity and characteristics. Hence the present investigation was undertaken to carry out a quantification study on the lectins from different species of *Brassica* genus, wild crucifers and other crops viz. *Allium cepa* and *Allium sativum*.

Materials and methods

Plant material included seeds and inflorescence of wild crucifers, vegetable *Brassicas* and other crops viz. *Allium cepa* and *Allium sativum*. Crop of oilseed *Brassicas*, vegetable *Brassicas*, wild crucifers, *Allium cepa* and *Allium sativum* were raised following recommended package of practices of Punjab Agricultural University, Ludhiana.

For carrying out haemagglutination assay, rabbit blood was procured from Rabbit Farm, Department of Livestock Production Management (LPM), Guru Angad Dev Veterinary and Animal Sciences University (GADVASU). For studying interaction of lectins with RBC's of diverse types, collected blood from various sources; human blood samples of ABO system were taken from Dayanand Medical College and Hospital (DMCH), Ludhiana. Blood samples of buffalo and goat were procured from Department of LPM, GADVASU.

Extract preparation:

The inflorescence tissue and defatted seed meal (0.5 g) was homogenized in 2 ml of 0.2 M Phosphate Buffered Saline (PBS), pH 7.4. The homogenate was centrifuged at 10,000 rpm for 20 minutes at 4 °C and the supernatant collected. The haemagglutination activity was estimated using the supernatants obtained from different extracts by the serial dilution method of Liener & Hill (1953). Protein content in the extracts was determined by method of Lowry *et al* (1951).

Results and discussion

Haemagglutination Activity of Lectins in Different Test Entries

The data for presence and absence of lectin activity in seeds and inflorescence of various test entries is summarized in Table 1. The total and specific activities are expressed as HU (haemagglutination units) per gram (seeds) and HU (haemagglutination units) per mg protein respectively.

Out of the test entries, nine entries were positive for lectin activity in seeds. The total lectin activity therein ranged from 80 to 800 HU/g (Table 2). *Diplotaxis muralis* (wild crucifer) registered highest lectin activity of 800 HU/g while GSC-6 (*Brassica napus*) and TL-15 (*Brassica rapa*) registered lowest lectin activity of 80 HU/g. Specific lectin activity ranged from 1.54 to 22.06 HU/mg protein with *Diplotaxis muralis* having maximum value of 22.06 HU/mg protein while *Diplotaxis assurgens* registered lowest value of 1.54 HU/mg protein (Table 2). All the test entries differed significantly with respect to hemagglutination activity (total and specific), *except* for *Lepidium sativum*, *Diplotaxis assurgens*, Turnip, GSC-6 and TL-15 which were at par with each other.

The inflorescence data revealed the presence of lectin in inflorescence of *Allium cepa* only with total and specific lectin activity of 640 HU/g fr.wt. and 68.80 HU/mg protein respectively. The data on lectin activity indicated the variation to be organ specific. Santos et al. (2009) reported variable amount of lectin activity in all extracts obtained from *Moringa oleifera* tissues (flowers, inflorescence rachis, seeds, leaf tissue,

fundamental tissue of stem and stem bark). The same has been observed for different tissues of *Dolichos lablab* (Rameshwaram and Nadimpalli, 2008).

Table 1 :Test entries checked for haemagglutination activity in seeds and inflorescence

Test entries	Seeds	Inflorescence
Oilseed brassicas		
RLM-619	-	-
RLC-1	-	-
PBR-210	-	-
PBR-97	-	-
BSH-1	-	-
TL-15	+	-
GSC-6	+	-
DLSC-2	-	-
PC-5	-	-
Vegetable brassicas and other crops		
Brocolli	-	-
Chinese cabbage	-	-
Punjab katki (cauliflower)	-	-
Radish	+	-
Turnip	+	-
Onion	-	+
***Wild* crucifers**		
Lepidium sativum	+	-
Sinapis alba	-	-
Capsella	-	-
Diplotaxis muralis	+	-
Diplotaxis assurgens	+	-
Diplotaxis gomezeampoi	+	-
Brassica tournifortii	+	-
Brassica fruticulosa	-	-
Brassica spinescens	-	-
Camelina	-	-

Table 2: Total and specific haemagglutination activity in seeds of test entries.

S.No	Test entries	Seeds	
		Total haemagglutination activity (HU/g)	**Specific haemagglutination activity (HU/mg protein)**
1	*Lepidium sativum*	120±0.0	3.37±0.9
2	*Brassica tournifortii*	480±0.0	9.41±1.2
3	*Diplotaxis assurgens*	120±0.0	1.54±0.6
4	*Diplotaxis gomezeampoi*	600±25.6	15.18±3.5
5	*Diplotaxis muralis*	**800**±0.0	**22.06**±3.1
6	*Raphanus sativus*	320±0.0	4.65±1.9
7	Turnip (*Brassica rapa)*	160±26.16	2.07±0.4
8	GSC-6 (*Brassica napus*)	80±0.0	2.35±0.5
9	TL-15 (*Brassica rapa*)	80±0.0	1.73±0.1

Anova Table

SOURCE	d.f.	M.S.	F-Ratio	CD(5%)
Replicates	1	10472.860	1.44	NS
Treatments	8	145803.70	20.05	189.928
Error	8	7272.7140		

Blood Group Specificity

The data on agglutination of erythrocytes from diverse sources, by seed lectins from various test entries is depicted in Table 3. For this, 2% RBC suspension was prepared from whole blood of rabbit, blood from A, B, O blood groups of human, buffalo blood and goat blood and assayed for agglutination with lectin preparation of test entries. Seed lectin from *Lepidium sativum* did not show positive agglutination with buffalo, AB and O blood groups while agglutinated RBC's from rabbit, goat, human A and B blood groups. Seed lectins from *Brassica tournifortii*, *Diplotaxis assurgens* and *Diplotaxis muralis* demonstrated positive agglutination with erythrocytes from human ABO system, rabbit, buffalo and goat, indicating that these lectins are blood group aspecific. Turnip, GSC-6 (*Brassica napus*) and TL-15 (*Brassica rapa*) seed lectins did not show agglutination with RBC's of human ABO system, buffalo and goat except for giving positive results with rabbit erythrocytes. Rabbit RBC's were ubiquitous in registering agglutination response with seed lectins from all test entries. Almost all the lectins studied so far, from diverse sources in nature, agglutinate rabbit RBC's but agglutination of RBC's from other sources (human A, B, O blood group RBC's, buffalo RBC's, goat

RBC's etc) is specific (Taniguchi et al., 1995; Ziska et al., 1982; Suseelan et al., 1997; Wang et al., 2004; Bashir et al., 2010). The affinity purified *Lepidium sativum* lectin agglutinated erythrocytes of animal origin and human erythrocytes from all blood group types (Ziska et al, 1982). The four Raphnin lectin isoforms purified by Taniguchi *et al* (1995) agglutinated rat erythrocytes, trysinized rabbit erythrocytes but did not agglutinate human A, B, O erythrocytes as well as horse, turkey, ox and chicken erythrocytes. The *Vigna mungo* lectins agglutinate trypsin treated rabbit erythrocytes but not the human erythrocytes of A, B and O groups (Suseelan et al, 1997). *Allium cepa* inflorescence lectin did not agglutinate erythrocytes of human ABO, buffalo and goat, however agglutinated rabbit erythrocytes only. The selectivity of different plant lectins for agglutinating erythrocytes from different sources could be based on their capacity to bind different carbohydrate structures on the cell membrane of erythrocytes (Vandenborre et al, 2011).

Table 3: Agglutination of the erythrocytes of various animal species by seed lectins from various test entries

S. No	Test entries	2 % RBCs suspension						
		Rabbit	Buffalo	Goat	Human Blood Group			
					A	B	A B	O
1	*Lepidium sativum*	+	-	+	+	+	-	-
2	*Brassica tournifortii*	+	+	+	+	+	+	+
3	*Diplotaxis assurgens*	+	+	+	+	+	+	+
4	*Diplotaxis gomezeampoi*	+	+	+	-	+	+	+
5	*Diplotaxis muralis*	+	+	+	+	+	+	+
6	Radish (*Raphanus sativus*)	+	+	-	+	+	+	+
7	Turnip	+	-	-	-	-	-	-
8	GSC-6 (*Brassica napus*)	+	-	-	-	-	-	-
9	TL-15 (*Brassica rapa*)	+	-	-	-	-	-	-

Conclusions

Our results show that the maximum lectin activity was registered in seeds of entries belonging to wild crucifers with *Diplotaxis muralis* showing the highest total lectin activity of 800 HU/ g. Other wild entries positive for presence of lectins in seeds include *Lepidium sativum*, *Brassica tournifortii*, *Diplotaxis assurgens* and *Diplotaxis gomezeampoi*. In general wild crucifers are resistant to attack of insect pests hence, these wild crucifers (*Diplotaxis muralis*, *Lepidium sativum*, *Brassica tournifortii*, *Diplotaxis assurgens*, *Diplotaxis gomezeampoi*) can be used in introgression of gene(s) for mustard aphid resistance in the background of commercial mustard genotypes. Alternatively, the genes of wild crucifers can be deployed in the transgenic technology which by all means, is a safe source of transgene (plant source). The significance of lectin gene for transgenic technology lies in the fact that it expresses edible protein which poses no threat to human health in transgenic plant.

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