Biocatalysis and Agricultural Biotechnology 19 (2019) 101121

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Purification and partial characterization of seed lectins from *Vicias* belonging to subgenus *Vicilla* section *Cracca*

Cristina Megias, Isabel Cortés-Giraldo, Julio Giron-Calle, Manuel Alaiz, Javier Vioque*

Food Phytochemistry Department, Instituto de la Grasa (C.S.I.C.), Campus Universidad Pablo de Olavide, Carretera de Utrera Km 1, 41013, Sevilla, Spain

ARTICLE INFO

Keywords:
Vicia
Lectins
N-Acetyl-galactosamine

ABSTRACT

Lectins from the seeds of ten *Vicias* belonging to section *Cracca* (*V. benghalensis*, *V. dasycarpa*, *V. monantha*, *V. villosa*, *V. cracca*, *V. vicioides*, *V. pseudocracca*, *V. disperma*, *V. tenuifolia* and *V. monardii*) have been purified by ultrafiltration and gel filtration chromatography, and characterized. All lectins belonged to the single-chain legume lectin group, having four subunits with molecular weights around 35–40 kDa, and a native molecular weight around 160–170 kDa. A dendogram based on amino acid composition matched the grouping based on quaternary structure. Agglutination assays indicated that affinity for N-acetyl-galactosamine was more frequent than affinity for glucose. N-acetyl-galactosamine is abundant in animal proteins such as mucins, and is part of the Tn antigen that has been related with malignancy, metastasis and bad prognosis in cancer. Hence, lectins from these *Vicia* seeds may be of interest for targeting cancerous cells and other animal cells that expose N-acetyl-galactosamine in their glycocalix.

1. Introduction

Lectins are defined as carbohydrate binding proteins of non-immune origin and without any enzymatic activity. Many plant lectins have been purified and characterized (Van Damme, Peumans, Barre & Rougé, 1998). The seeds of some legumes are rich in lectins, which have been purified from commercial legumes such as soybean, peanut, broad bean, pea, and lentil (Rüdiger and Gabius, 2001). Different functions have been proposed for seed lectins, including defense against predators (Vasconcelos and Oliveira, 2004; Vandenborre et al., 2011), acting as storage proteins (Van Damme, Peumans, Barre & Rougé, 1998), and stabilization of protein bodies (Rüdiger and Gabius, 2001). Lectins in legumes are classified into two major groups according to their structure: the single-chain and two-chain lectin groups (Loris et al., 1998). Single-chain lectins are composed of four identical or very similar peptides of around 30 kDa, while two chain lectins are made of two light and two heavy subunits of around 6 and 17–20 kDa, respectively (Van Damme, Peumans, Barre and Rougé, 1998). Lectins in soybean and peanut belong to the first group while those in lentil and pea belong to the second (Rüdiger and Gabius, 2001).

The genus *Vicia* includes both single and double chain lectins. For instance, lectins in *V. faba* and *V. ervilia* belong to the two-chain group (Hemperly et al., 1979; Fornstedt and Porath, 1975) while lectins in *V. villosa* (Grubhoffer et al., 1981), *V. unijuga* (Yanagi et al., 1990), and *V. graminea* (Prigent and Bourrillon, 1976) belong to the single-chain group. Sometimes both types of lectins are present in the same species, e.g. lectins in *V. cracca* (Baumann et al., 1982). Lectins also differ in their affinity for sugars. Thus, single-chain lectins show, in general, higher affinity for N-acetyl-galactosamine (GalNAc), while two-chain lectins prefer mannose and glucose. For instance, the *V. faba* two-chain lectin shows affinity for mannose and glucose, while the single-chain lectins in *V. villosa*, *V. graminea*, and *V. cracca* display a higher affinity for GalNAc (Van Damme et al., 1998). The lectin from *V. villosa* is particularly well known because it has been sequenced (Osinaga et al., 1997) and its crystal structure has been determined (Babino et al., 2003). It shows affinity for GalNAc that is part of the Tn cancer antigen (Fuster and Esko, 2005), and the crystal structure of the lectin-Tn antigen complex has been analyzed (Babino et al., 2003).

The Tn antigen is formed by GalNAc glycosylation of hydroxyl groups belonging to serine or threonine residues in proteins. This antigen can be found in secreted proteins such as mucins (Hollingsworth and Swanson, 2004) and in cell surface proteins (Fuster and Esko, 2005). In healthy cells, GalNAc is capped by addition of other sugars, but in malignant cells the Tn antigen is not capped due to an incomplete elongation of the O-glycan. Different studies have reported a positive correlation between cancer aggressiveness and the presence of the Tn antigen (Springer, 1997). The lectin from *V. villosa* has been used to quantify the Tn antigen in sera from cancer patients (Osinaga et al.,

---

* Corresponding author.
E-mail address: jvioque@cica.es (J. Vioque).

https://doi.org/10.1016/j.bcab.2019.101121
Received 31 January 2019; Received in revised form 19 March 2019; Accepted 1 April 2019

1996; Konska et al., 2006). Recently, a label-free biosensor has been developed using the lectin from *V. villosa* for detection of the Tn antigen (Silva and Rangel, 2017). This lectin has also been used to discriminate between different types of neurons (Ojima et al., 1995).

V. villosa is grown for feed and as green manure, and belongs to subgen. *Vicilla* sect. *Cracca* (Schaefer et al., 2012). Other taxonomically related *Vicia,* also belonging to sect. *Cracca,* are of interest from an agricultural and nutritional point of view. Thus, *V. dasycarpa*, *V. monantha*, *V. benghalensis*, *V. cracca*, and *V. tenuifolia* are or have been cultivated for food, feed or as green manure. Taxonomically related to these species are the wild species *V. disperma*, *V. pseudocracca*, *V. monardii,* and *V. vicioides* (Romero-Zarco and AuthorAnonymous, 1999). These *Vicia* species may represent new sources of lectins with biochemical and technological applications similar to those described for the lectin isolated from *V. villosa*. The objective of this work was to purify and characterize the lectins from these nine *Vicia* species related to *V. villosa* in order to determine whether these lectins may also be of interest because of potential biochemical and technological applications.

2. Material and methods

2.1. Materials

Bromophenol blue, Coomassie brilliant blue G, diethyl ethoxy-methylene-malonate, D-L-$\alpha-$aminobutyric acid, glutaraldehyde, sodium azide, trypsin, *Lens culinaris* and *Canavalia ensiformis* lectins were from Sigma Aldrich. All other reagents were of analytical grade. *V. villosa* seeds (INIA-1383) were provided by CRF-INIA (Madrid, Spain). All other *Vicia* seeds were collected from the wild in southern Spain.

2.2. Purification of lectins

A suspension of seed flour in water 1/10 (w/v) was stirred for 30 min at room temperature while pH was kept at 4. The solid residue after centrifugation at $10{,}000 \times g$ was extracted once more. The combined extracts containing the solubilized lectins were concentrated by ultrafiltration using an Amicon cell filtration unit with a 3 kDa cut-off membrane, and applied to a Superose 12 10/300 (GE Life Sciences) gel filtration column mounted on an AKTA Purifier system (GE Life Sciences). Injection volume was 1 mL, and 50 mM Na_2PO_4, 0.5 M NaCl pH 7 buffer was used as eluent. Elution of the lectins was followed by SDS-PAGE of the collected fractions (0.5 mL), and those that did not contain pure lectin were chromatographed again.

2.3. SDS-PAGE

Protein extracts were adjusted to 2 mg protein/mL, mixed (1:1 v/v) with solubilisation buffer (80 mM Tris, 0.57% EDTA, 0.26% DTT, 3.3% SDS, 0.008% bromophenol blue, 20% sucrose, pH 6.8), and heated at 100 °C for 10 min. Tricine-SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Schägger and von Jagow (1987) at a constant voltage of 60 V for the stacking gel, and 120 V for the separation gel, with 25 mM Tris, 190 mM glycine, 0.1% SDS pH 8 as running buffer. Gels were fixed in 20% methanol, 8% acetic acid for 15 min before staining using 0.25% Coomassie brilliant blue G in 45% methanol 10% acetic acid. Molecular masses were determined using the low molecular weight standards from GE Healthcare Life Sciences.

2.4. Native PAGE

Native PAGE was carried out using the Mini-PROTEAN TGX (4–20%) precast gels from BIO-RAD (CA, U.S.A.). Protein extracts were adjusted to 2 mg protein/mL, mixed (1:1 v/v) with solubilisation buffer (running buffer containing 0.5% bromophenol blue, 20% sucrose). Native PAGE was performed at a constant voltage of 200 V with 25 mM Tris, 190 mM glycine pH 8 as running buffer. Gels fixation and staining was as for SDS-PAGE. Molecular masses were determined using the high molecular weight standards from Pharmacia LKB Biotechnology.

2.5. Amino acid analysis

Samples were hydrolyzed by incubation in 6 N HCl at 110 °C for 20 h. Amino acids were determined after derivatization with diethyl ethoxymethylenemalonate by high-performance liquid chromatography (HPLC) according to the method described by Alaiz et al. (1992) using D-L-$\alpha-$aminobutyric acid as internal standard. Samples (20 µL) were injected in a reversed-phase column (Novapack C_{18}, 300 × 3.9 mm i.d., 4 µm, Waters) that was kept at 18 °C. Tryptophan was determined by HPLC after basic hydrolysis according to Yust et al. (2004).

2.6. Erythrocytes agglutination assay

Glutaraldehyde-fixed, trypsinized rat erythrocytes for the agglutination assays were prepared as follows. The pellet resulting from centrifugation of rat blood at 500 g for 10 min was washed three times by resuspension in PBS and centrifugation (500 g 10 min). The resulting pellet was resuspended 1/10 (v/v) in PBS containing 0.5% (v/v) glutaraldehyde and incubated in a shaker for 1 h at room temperature. Erythrocytes were recovered by centrifugation and washed with PBS three times as previously described, and were resuspended in PBS (20% v/v) containing sodium azide (0.1% w/v). The fixed erythrocytes (2 mL) were trypsinized by incubation with trypsin (2 mg) for 30 min at 37 °C, and washed with PBS three times as described above.

Agglutination assays were carried out by incubating these trypsinized, glutaraldehyde fixed rat erythrocytes (40 µL, 4% w/v in PBS buffer) in the presence of increasing concentrations of lectin in 96 U shape well microplates for 1 h at room temperature. Agglutinating activity was categorized as negative or positive for each well. *C. ensiformis* lectin (1 µg/well) was used as a positive control, and incubations of erythrocytes with no lectin added were used as negative control. Minimun lectin concentration that yield agglutination was used to assay agglutination inhibition by sugars. Decreasing amounts of galNAc and glucose where employed to determine the minimum concentration of sugar that inhibits the agglutination by lectins.

2.7. Statistical analysis

Cluster analysis of *Vicia* lectins was performed according to the furthest neighbor method based on a euclidean distance matrix using the Stagraphics 5.1 software.

3. Results and discussion

3.1. Purification of Vicia lectins

Lectins in leguminous seeds are albumins according to the classic Osborne protein classification because they are soluble in plain water, in contrast to storage proteins (globulins) that are soluble in saline solutions but not in water. Hence, lectins in *Vicia* seeds were extracted in water at pH 4, corresponding to the isoelectric point of globulins. Fig. 1A illustrates these differences in solubility by showing as an example two different extracts from one of the *Vicia* species that were used for extraction of lectins, *V. benghalensis*. While extraction using SDS-PAGE running buffer (Fig. 1, left line) yields the most intense protein bands in the molecular weight range between 43 and 94 kDa corresponding to globulins, extraction in water at pH 4 (Fig. 1, right line) yields peptides around 30 kDa corresponding to lectins.

Because components that could interfere with the agglutination assay are also extracted with water, all lectin extracts were further purified by gel filtration chromatography. These potentially interfering

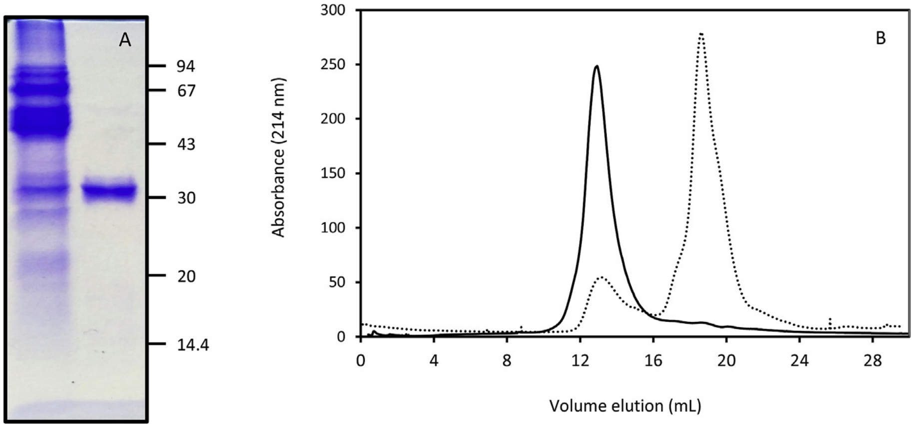

Fig. 1. A) SDS-PAGE of *V. benghalensis* seed proteins extracted using SDS-PAGE running buffer (left line) or in water adjusted to pH 4 (right line). Molecular weight markers are indicated on the rigth. **B)** Superose 12 gel filtration profile of the original *V. benghalensis* pH 4 water extract (dashed line) and after purification by using the same gel filtration procedure (full line).

components include polyphenols and sugars. Thus, soluble sugars can inhibit lectin agglutination by binding to the lectin active site, while polyphenols can actually have agglutinating activity by themselves which might lead to overestimate agglutination (Cortés-Giraldo et al., 2012). Water extracts were applied to a gel filtration Superose 12 column, and fractions were pooled and concentrated to yield a chromatographically pure lectin preparation as shown in Fig. 1B for *V. benghalensis*.

The molecular weight of the purified lectin subunits is around 35–40 kDa, indicating that they belong to the single-chain legume lectin group (Fig. 2A). This confirms previous reports concerning *V. villosa* (Grubhoffer et al., 1981) and *V. cracca* (Rüdiger, 1977). *V. villosa* and *V. monardii* showed two different bands corresponding to 37 and 40 kDa, and 35 and 36 kDa, respectively. Native PAGE was carried out in order to further confirm the single-chain nature of the lectins (Fig. 2B). A similar molecular weight of around 160–170 kDa was found in all lectins, similar to that reported for other single-chain lectins in *Vicia* (Grubhoffer et al., 1981; Rüdiger, 1977).

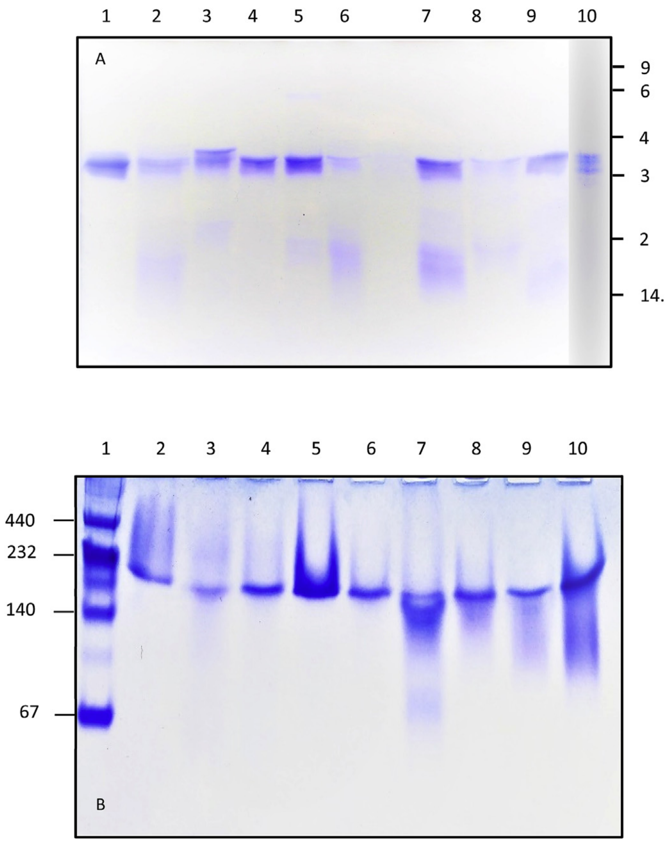

Fig. 2. A) SDS-PAGE of purified *Vicia* lectins: 1) *V. benghalensis,* 2) *V. dasycarpa*, 3) *V. villosa*, 4) *V. monantha*, 5) *V. cracca*, 6) *V. tenuifolia*, 7) *V. disperma*, 8) *V. vicioides*, 9) *V. pseudocracca*, 10) *V. monardii*. **B)** Native PAGE of purified *Vicia* lectins. 1) *V. benghalensis*, 2) *V. dasycarpa*, 3) *V. villosa*, 4) *V. monantha*, 5) *V. cracca*, 6) *V. monardii*, 7) *V. disperma*, 8) *V. vicioides*, 9) *V. pseudocracca*. Molecular weight standards are indicated on the side (kDa).

The amino acid composition of the purified lectins shows that the most abundant residues in these proteins are Asx, Glx, Thr, Lys, and Leu (Table 1). The last column represents the mean amino acid composition for these ten lectins, and is plotted in Fig. 3 as compared to the mean amino acid composition of a group of two-chain lectins that were purified from the seeds of *Pisum sativum*, *Lens culinaris*, *Vicia faba*, *Lathyrus sativus*, and *Lathyrus cicera* (unpublished results). All these legumes are included, together with *Vicia*, in Tribe *Fabeae*. Significative differences ($P < 0.001$) where observed in the amounts of Asp, Ser, His, Gly, Thr, Arg, Ala, Val, Met, Ile, Trp and Lys between the two types of legume lectins. These differences between the single-chain and two-chain lectins were highlighted in a dendogram that was constructed based on the amino acid composition using the furthest neighbor method (Fig. 4). According to this dendogram, the purified *Vicia* single-chain lectins are grouped in a single clade, clade A, while the two chain lectins of other legumes belonging to Tribe *Fabeae* are arranged in clade B. Clade C includes taxonomically distant legumes with single-chain type lectins. Clade D is a higher clade including clades C and A. Thus, the arrangement provided by the dendogram based on amino acid composition discriminates between single-chain and two-chain lectins.

3.2. *Erythrocytes agglutinating activity*

Table 2 shows the agglutinating activity of the purified *Vicia* lectins, and includes also the agglutinating activity of the lectins from *Lens culinaris* and *Canavalia ensiformis* for comparison. All *Vicia* lectins, except for those corresponding to *V. pseudocracca* and *V. monardii*, presented agglutinating activity at a concentration of 10 µg lectin/mL or bellow, which was also the case of the lectins from *Lens culinaris* and *Canavalia ensiformis* (Table 2). The activity was lower in the lectins belonging to *V. pseudocracca* and *V. monardii*, with a minimum agglutinating lectin concentration of 59 and 140 µg/mL, respectively.

In general, binding to sugars by two-chain and one-chain lectins in Tribe *Fabeae* is specific for glucose and for GalNAc, respectively. Thus, the two-chain lectins in *V. faba*, *L. culinaris*, and *P. sativum* are specific for glucose, while the one-chain lectin in *V. villosa* is specific for GalNAc (Van Damme, Peumans, Barre & Rougé, 1998). The inhibition of agglutinating activity in the presence of glucose and GalNAc as shown in Table 2 roughly follows this pattern. Thus, as previously reported for *V. villosa*, the agglutinating activity of the single-chain lectins from *V. benghalensis*, *V. dasycarpa*, *V. monantha*, *V. villosa*, *V. pseudocracca* and *V. monardii* was specific for GalNAc, while lectins from *V. cracca*, *V.*

Table 1

Amino acids composition (g/100 g lectin) of purified *Vicia* seed lectins. Results are the average ± standard deviation of two independent determinations.

	Vicia benghalensis	Vicia dasycarpa	Vicia monantha	Vicia villosa	Vicia cracca	Vicia vicioides	Vicia pseudocracca	Vicia disperma	Vicia tenuifolia	Vicia monardii	x ± sd
Asx[a]	12.9 ± 0.01	14.2 ± 0.01	13.2 ± 0.10	15.4 ± 0.60	15.1 ± 0.04	14.1 ± 0.20	13.1 ± 0.02	13.2 ± 0.05	13.3 ± 0.18	15.4 ± 0.04	14.0 ± 0.94
Glx[b]	9.2 ± 0.03	11.6 ± 0.04	10.7 ± 0.34	8.0 ± 0.35	9.6 ± 0.06	10.0 ± 0.04	9.5 ± 0.01	9.6 ± 0.02	16.1 ± 0.04	12.6 ± 0.00	10.7 ± 2.17
Ser	7.7 ± 0.04	7.2 ± 0.02	7.2 ± 0.02	6.9 ± 0.05	7.6 ± 0.04	7.2 ± 0.08	7.9 ± 0.00	7.8 ± 0.03	6.4 ± 0.01	6.3 ± 0.01	7.2 ± 0.53
His	2.4 ± 0.01	2.4 ± 0.08	2.6 ± 0.01	3.0 ± 0.01	2.6 ± 0.03	2.6 ± 0.04	2.2 ± 0.01	2.3 ± 0.02	1.9 ± 0.03	2.4 ± 0.02	2.4 ± 0.28
Gly	3.4 ± 0.01	3.7 ± 0.02	4.1 ± 0.02	6.4 ± 0.09	3.8 ± 0.03	3.5 ± 0.01	3.5 ± 0.01	3.7 ± 0.01	6.2 ± 0.03	6.4 ± 0.02	4.5 ± 1.22
Thr	8.4 ± 0.01	8.2 ± 0.02	8.7 ± 0.05	6.6 ± 0.06	8.5 ± 0.04	8.3 ± 0.06	8.9 ± 0.00	7.8 ± 0.03	9.0 ± 0.01	8.2 ± 0.01	8.2 ± 0.66
Arg	2.8 ± 0.02	3.6 ± 0.10	3.9 ± 0.03	4.1 ± 0.04	3.4 ± 0.03	3.5 ± 0.03	4.0 ± 0.03	3.6 ± 0.00	3.9 ± 0.06	4.2 ± 0.05	3.7 ± 0.40
Ala	5.2 ± 0.01	5.9 ± 0.05	5.7 ± 0.00	5.2 ± 0.00	5.0 ± 0.07	4.9 ± 0.06	4.9 ± 0.07	5.9 ± 0.01	8.6 ± 0.00	7.5 ± 0.00	5.9 ± 1.16
Pro	3.3 ± 0.08	3.4 ± 0.11	3.0 ± 0.12	3.3 ± 0.08	3.2 ± 0.17	2.8 ± 0.02	2.7 ± 0.01	2.7 ± 0.00	2.7 ± 0.00	2.7 ± 0.06	3.0 ± 0.29
Tyr	3.3 ± 0.04	2.1 ± 0.01	2.3 ± 0.00	5.0 ± 0.01	2.2 ± 0.02	2.3 ± 0.03	3.1 ± 0.02	3.3 ± 0.00	1.9 ± 0.01	2.5 ± 0.01	2.8 ± 0.88
Val	8.0 ± 0.10	5.9 ± 0.05	7.0 ± 0.19	5.6 ± 0.02	7.1 ± 0.08	7.7 ± 0.01	6.4 ± 0.02	6.8 ± 0.01	4.8 ± 0.02	4.8 ± 0.00	6.4 ± 1.07
Met	0.0 ± 0.01	0.2 ± 0.00	0.1 ± 0.01	0.1 ± 0.02	0.4 ± 0.00	0.6 ± 0.01	0.1 ± 0.00	0.0 ± 0.00	0.1 ± 0.00	0.3 ± 0.01	0.2 ± 0.18
Cys	0.1 ± 0.01	0.2 ± 0.01	0.5 ± 0.00	1.0 ± 0.15	0.5 ± 0.00	0.3 ± 0.03	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.3 ± 0.03	0.3 ± 0.30
Ile	5.5 ± 0.02	6.6 ± 0.02	5.4 ± 0.10	5.2 ± 0.20	5.6 ± 0.00	6.5 ± 0.02	6.4 ± 0.00	5.9 ± 0.01	3.2 ± 0.04	3.8 ± 0.03	5.4 ± 1.07
Trp	1.9 ± 0.06	2.1 ± 0.05	2.0 ± 0.06	2.2 ± 0.06	2.2 ± 0.08	2.6 ± 0.02	2.7 ± 0.03	2.6 ± 0.04	2.4 ± 0.00	2.6 ± 0.00	2.3 ± 0.27
Leu	9.4 ± 0.02	7.5 ± 0.07	8.2 ± 0.07	9.8 ± 0.13	8.6 ± 0.12	7.6 ± 0.05	8.0 ± 0.00	7.5 ± 0.01	5.3 ± 0.02	6.7 ± 0.02	7.9 ± 1.24
Phe	9.2 ± 0.02	7.1 ± 0.03	8.0 ± 0.15	6.0 ± 0.02	7.6 ± 0.12	8.1 ± 0.02	8.7 ± 0.03	8.4 ± 0.04	3.3 ± 0.07	4.0 ± 0.03	7.0 ± 1.90
Lys	7.3 ± 0.02	8.2 ± 0.07	7.5 ± 0.03	6.4 ± 0.02	7.0 ± 0.06	7.4 ± 0.06	7.8 ± 0.01	8.9 ± 0.05	11.2 ± 0.09	9.4 ± 0.01	8.1 ± 1.34

[a] Asp + Asn.

[b] Glu + Gln.

disperma and *V. tenuifolia* showed a similar specificity for GalNAc and glucose, and only the lectin from *V. vicioides* was more sensitive to inhibition by glucose.

Tribe *Fabeae* of Leguminosae includes genera *Vicia*, *Lathyrus*, *Pisum*, *Lens* and *Vavilovia*. Until now, most of the lectins purified from the seeds of legumes belonging to this Tribe were of the two-chain type, including the lectins from *P. sativum*, *L. culinaris*, *V. faba, V. cracca*, and several *Lathyrus* species (Van Damme, Peumans, Barre and Rougé, 1998). On the contrary, single-chain lectins were only found in some species of genus *Vicia* such as *V. villosa* and *V. cracca* (Grubhoffer et al., 1981; Rüdiger, 1977). Our results showing the presence of single-chain lectins in all of the ten *Vicia* species that have been studied indicate that single-chain lectins are more common in Tribe *Fabeae* than previously believed. This does not exclude the potential presence of two-chain lectins in these seeds in lower amounts than single-chain lectins, as has been described in *V. cracca* (Baumann et al., 1982; Rüdiger, 1977). The quaternary structure and molecular weight of the lectins purified from *V. cracca* and *V. villosa* are consistent with those previously described (Grubhoffer et al., 1981; Rüdiger, 1977). The preference for GalNAc in the lectins purified from *V. villosa*, *V. cracca* and *V. graminea* is also consistent with previous reports (Tollefsen & Kornfeld, 1983, 1984; Wu, 2005).

Although GalNAc is found in animal cells in many glycoproteins such as mucins (Gill et al., 2011), it was not until few years ago that GalNAc was found in plant cells, namely in *Arabidopsis* cells cultures (Alonso et al., 2010). It is not known whether GalNAc is incorporated into glycans or other biomolecules in these cells (Bar-Peled & O'Neill, 2011). The presence of the Tn antigen in tumors correlates with cancer cell proliferation, metastasis, and a bad prognosis (Springer, 1997; Ju et al., 2013). Some methods to detect and quantify the Tn antigen in malignant cells are based on the affinity of lectins for GalNAc (Kakeji et al., 1991). Thus, the lectin from *V. villosa* has been used to study the expression of the Tn antigen in colon (Itzkowitz et al., 1989), breast (Konska et al., 2006) and ovarian tumors (Chen et al., 2013). Interestingly, the lectin from *Morus nigra* covalently linked to a porphyrin has been used to target the Tn antigen in order to kill leukemia cells by photochemotheraphy (Poiroux et al., 2011). Some of the *Vicia* lectins that are presented in this article may also represent a useful tool for targeting GalNAc, especially those that present an affinity for this sugar even higher than the lectin from *V. villosa*. That is the case for instance of the lectins from *V. benghalensis* and *V. monantha*, with an affinity for GalNAc which is more than five times higher than the affinity of the lectin from *V. villosa*. *V. benghalensis* and *V. monantha* are two forage crops that have good agronomic characteristics including a high tolerance to biotic and abiotic stress (Francis et al., 2000), fast growth, and high yield (Bryant et al., 2011). In conclusion, purification and characterization of the lectins from ten species of *Vicia* species belonging to sect. *Cracca* subgen. *Vicilla* supports the view that this section is rich in single-chain lectins. Some of these lectins bind to GalNAc with high affinity. Some of these *Vicias* may be of interest as sources of lectins for research in glycobiology with potential biomedical applications for targeting the Tn antigen.

Acknowledgements.

This work was carried out with the financial support of Junta de Andalucía (Spain) to the Laboratory of Bioactive and Functional Components of Plant Products (Instituto de la Grasa, C.S.I.C.). Cristina Megias was recipient of a JAE-Doc (C.S.I.C.) contract from the "Junta para la Ampliación de Estudios" program (cofinanced by the European Social Fund). Isabel Cortés-Giraldo was recipient of a JAE-Pre (C.S.I.C) fellowship from the "Junta para la Ampliación de Estudios" program (cofinanced by the European Social Fund). Thanks are due to Centro de Recursos Fitogenéticos seed bank (CRF-INIA, Madrid, Spain) for providing *V. villosa* seeds.

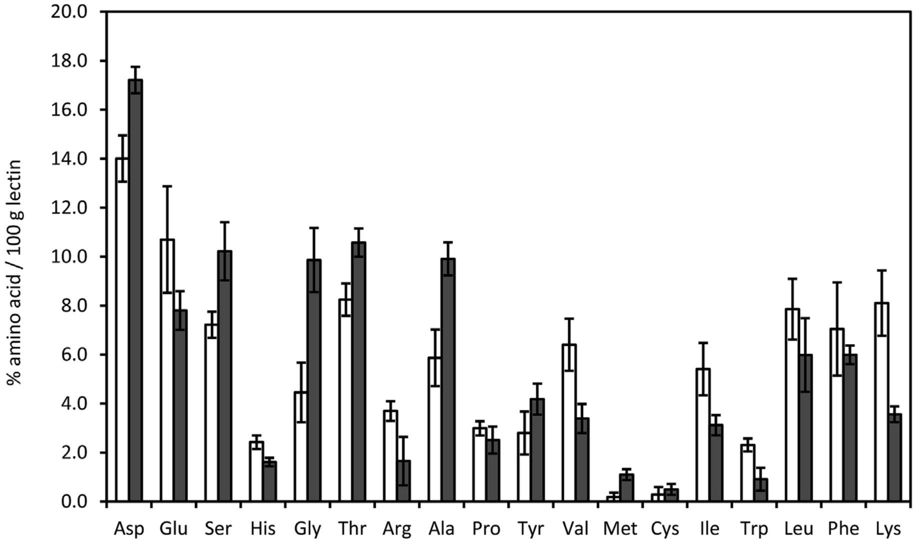

Fig. 3. Average amino acid composition of the one-chain lectins from *Vicia* species in Table 1 (open bars), and two-chain lectins from *Pisum sativum*, *Lens culinaris*, *Lathyrus cicera*, *Lathyrus sativus* and *Vicia faba* (dark bars, unpublished results).

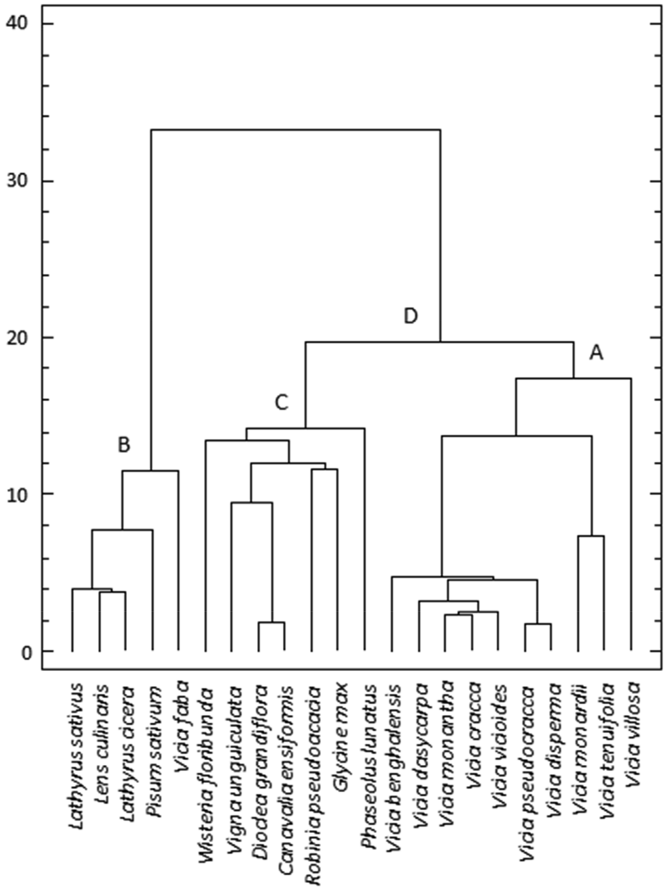

Fig. 4. Dendogram produced by cluster analysis of the amino acid composition of different one-chain and two-chain legume lectins. Amino acid composition of *Canavalia ensiformis* (ID, 2098436), *Phaseolus vulgaris* (ID 3891966), *Phaseolus lunatus* (ID 8920387), *Dioclea grandiflora* (ID 5107577), *Robinia pseudoacacia*, (ID 538529), *Vigna unguiculata* (ID 388103), *Wisteria floribunda* (ID 1064245663) were calculated from their protein sequence. The amino acid composition of *Vicia faba*, *Pisum sativum*, *Lathyrus sativus*, *Lathyrus cicera* and *Lens culinaris* correspond to unpublished results.

Table 2
Agglutinating activity of the lectins purified from *Vicia*, *Lens culinaris* and *Canavalia ensiformis*, and inhibition by GalNAc and glucose. Agglutinating activity of lectins is expressed as the minimum concentration of lectins (μg/mL) that cause agglutination of a 4% suspension of red blood cells. The inhibitory activity of the sugars is expressed as the minimum concentration of sugars (mM) that cause inhibition of agglutination. Results are the average of two determinations.

	[Lectin] (μg/mL)	GalNAc (mM)	Glucose (mM)
V. benghalensis	6.0	0.9	16.5
V. dasycarpa	9.0	3.6	65.0
V. monantha	5.6	0.9	65.0
V. villosa	3.4	5.0	33.0
V. cracca	4.0	14.4	16.5
V. vicioides	5.6	28.8	8.2
V. pseudocracca	58.8	0.9	33.0
V. disperma	3.6	3.6	6.7
V. tenuifolia	10.4	7.2	16.5
V. monardii	140.0	57.6	320.0
L. culinaris	4.0	90.4	6.7
C. ensiformis	8.0	90.4	6.7

References

Alaiz, M., Navarro, J.L., Giron, J., Vioque, E., 1992. Amino acid analysis by high-performance liquid chromatography after derivatization with diethylethoxymethylenemalonate. J. Cromatogr. 591, 181–186.

Alonso, A.P., Piasecki, R.J., Wang, Y., LaClair, R.W., Shachar-Hill, Y., 2010. Quantifying the labeling and the levels of plant cell wall precursors using ion chromatography tandem mass spectrometry. Plant Physiol. 153, 915–924.

Babino, A., Tello, D., Rojas, A., Bay, S., Osinaga, E., Alzari, P.M., 2003. The crystal structure of a plant lectin in complex with the Tn antigen. FEBS Lett. 536, 106–110.

Bar-Peled, M., O'Neill, M.A., 2011. Plant nucleotide sugar formation, interconversion, and salvage by sugar recycling. Annu. Rev. Plant Biol. 62, 127–155.

Baumann, C.M., Strosberg, D., Rüdiger, H., 1982. Purification and characterization of a mannose/glucose-specific lectin from *Vicia cracca*. Eur. J. Biochem. 122, 105–110.

Bryant, J.A., et al., 2011. In: Kole, C. (Ed.), Wild Crop Relatives and Breeding Resouces, Legume Crops and Forages. Springer-erlag.

Chen, K., Gentry-Maharaj, A., Burnell, M., Steentoft, C., Marcos-Silva, L., Mandel, U., Jacobs, T., Dawnay, A., Menon, U., Blixt, O., 2013. Microarray glycoprofiling of CA125 improves differential diagnosis of ovarian cancer. J. Prot. Res. 12, 1408–1418.

Cortés-Giraldo, I., Girón-Calle, J., Alaiz, M., Vioque, J., Megías, C., 2012. Hemagglutinating activity of polyphenols extracts from six grain legumes. Food Chem. Toxicol. 50, 1951–1954.

Fornstedt, N., Porath, J., 1975. Characterization studies on a new lectin found in seeds of Vicia ervilia. FEBS Lett. 57, 187–191.

Francis, C.M., Enneking, D., el Moneim, A.M., 2000. When and where will vetches have an impact as grain legumes? Curr. Plant Sci. Biotechnol. Agric. 34, 375–384.

Fuster, M.M., Esko, J.D., 2005. The sweet and sour of cancer: glycans as novel therapeutic targets. Nat. Rev. Canc. 5, 526–542.

Gill, D.J., Clausen, H., Bard, F., 2011. Location, location, location: new insights into *O*-GalNAc protein glycosylation. Trends Cell Biol. 21, 149–158.

Grubhoffer, L., Tichá, M., Kocourek, J., 1981. Isolation and properties of a lectin from the seeds of hairy vetch (*Vicia villosa* Roth). Biochem. J. 195, 623–626.

Hemperly, J.J., Hopp, T.P., Becker, J.W., Cunningham, B.A., 1979. The chemical characterization of favin, a lectin isolated from *Vicia faba*. J. Biol. Chem. 254, 6803–6810.

Hollingsworth, M.A., Swanson, B.J., 2004. Mucins in cancer: protection and control of the cell surface. Nat. Rev. Canc. 4, 45–60.

Itzkowitz, S.H., Yuan, M., Montgomery, C.K., Kjeldsen, T., Takahashi, H.K., Bigbee, W.L., Kim, Y.S., 1989. Expression of Tn, sialosyl-Tn, and T antigens in human colon cancer. Cancer Res. 49, 197–204.

Ju, T., Wang, Y., Aryal, R.P., Lehoux, S.D., Ding, X., Kudelka, M.R., Cutler, C., Zeng, J., Wang, J., Sun, X., Heimburg-Molinaro, J., Smith, D.F., Cummings, R.D., 2013. Tn and sialyl-Tn antigens, aberrant *O*-glycomics as human disease markers. Proteonomics Clin. Appl. 7, 618–631.

Kakeji, Y., Tsujitani, S., Mori, M., Maehara, Y., Sugimachi, K., 1991. Helix-pomatia agglutinin binding-activity is a predictor of survival-time for patients with gastric-carcinoma. Cancer 68, 2438–2442.

Konska, G., Guerry, M., Caldefie-Chezet, F., De Latour, M., Guillot, J., 2006. Study of the expression of Tn antigen in different types of human breast cancer cells using VVA-B-4 lectin. Oncol. Rep. 15, 305–310.

Loris, R., Hamelryck, T., Bouckaert, J., Wyns, L., 1998. Legume lectin structure. Biochim. Biophys. Acta 1383, 9–36.

Ojima, H., Kuroda, M., Ohyama, J., Kishi, K., 1995. 2 classes of cortical-neurons labeled with Vicia-villosa lectin in the Guinea-pig. Neuroreport 6, 617–620.

Osinaga, E., Babino, A., Grosclaude, J., Cairoli, E., Batthyany, C., Bianchi, S., Signorelli, S., Varangot, M., Muse, I., Roseto, A., 1996. Development of an immuno-lectin-enzymatic assay for the detection of serum cancer-associated glycoproteins bearing Tn determinant. Internet J. Oncol. 8, 401–406.

Osinaga, E., Tello, D., Batthyany, C., Bianchet, M., Tavares, G., Durán, R., Cerveñansky, C., Camoin, L., Roseto, A., Alzari, P.M., 1997. Amino acid sequence and three-dimensional structure of the Tn-specific osilectin B4 from Vicia villosa. FEBS Lett. 412, 190–196.

Poiroux, G., Pitie, M., Culerrier, R., Lafont, E., Ségui, B., Van Damme, E.J.M., Peumans, W.J., Bernadou, J., Levade, T., Rougé, P., Barre, A., Benoist, H., 2011. Targeting of T/Tn antigens with a plant lectin to kill human leukemia cells by photochemotherapy. PLoS One 6 (8), e23315.

Prigent, M.J., Bourrillon, R., 1976. Purification and characterization of a lectin (plant hemagglutinin) with N blood groups specificity from *Vicia graminea* seeds. Biochim. Biophys. Acta 420, 112–121.

Romero-Zarco, C., 1999. Vicia. In: In: Talavera, S., Aedo, C., Castroviejo, S., Romero-Zarco, C., Sáez, L., Salgueiro, F.J. (Eds.), Flora Ibérica, vol. vol. II Velayos, M. CSIC, Madrid).

Rüdiger, H., 1977. Purification and properties of blood-group-specific lectins from *Vicia cracca*. Eur. J. Biochem. 72, 317–322.

Rüdiger, H., Gabius, H.-J., 2001. Plant lectins: occurrence, biochemistry, functions and applications. Glycoconj. J. 18, 589–613.

Schaefer, H., Hechenleitner, P., Santos-Guerra, A., Menezes de Sequeira, M., Pennington, R.T., Kenicer, G., Carine, M.A., 2012. Systematics, biogeography, and character evolution of the legume tribe Fabeae with special focus on the middle-Atlantic island lineages. BMC Evol. Biol. 12, 250.

Schägger, H., von Jagow, G., 1987. Tricine-sodium dodecyl sulfate polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Anal. Biochem. 166, 368–379.

Silva, M.L.S., Rangel, M.G.H., 2017. A *Vicia villosa* agglutinin biosensor for cancer-associated Tn antigen. Sensor. Actuator. B Chem. 252, 777–784.

Springer, G., 1997. Immunoreactive T and Tn epitopes in cancer diagnosis, prognosis, and immunotherapy. J. Mol. Med. 75, 594–602.

Tollefsen, S.E., Kornfeld, R., 1983. The B4 lectin from Vicia villosa seeds interatcs with N-acetylgalactosamine residues alpha-linked to serine or threonine residues in cell surface glycoproteins. J. Biol. Chem. 258, 5172–5176.

Tollefsen, S.E., Kornfeld, R., 1984. The B4 lectin from Vicia villosa seeds interacts with N-acetylgalactosamine residues on erythrocytes with blood group Cad specificity. Biochem. Biophys. Res. Commun. 123, 1099–1106.

Van Damme, E.J.M., Peumans, W.J., Barre, A., Rougé, P., 1998. Plant lectins: a composite of several distinct families of structurally and evolutionary related proteins with diverse biological roles. Crit. Rev. Plant Sci. 17, 575–692.

Vandenborre, G., Smagghe, G., Van Damme, E.J.M., 2011. Plant lectins as defense proteins against phytophagous insects. Phytochemistry 72, 1538–1550.

Vasconcelos, I.M., Oliveira, J.T.A., 2004. Antinutritional properties of plant lectins. Toxicon 44, 385–403.

Wu, A.M., 2005. Polyvalency of Tn (GalNAca→Ser/Thr) glycotope as a critical factor for Vicia villosa B4 and glycoprotein interactions. FEBS Lett. 562, 51–58.

Yanagi, K., Ohyama, K., Yamakawa, T., Hashimoto, K., Ohkuma, S., 1990. Purification and characterization of anti-N lectin from *Vicia unijuga* leaves. Int. Jr. Biochem. 22, 43–52.

Yust, M.M., Pedroche, J., Girón-Calle, J., Vioque, J., Millán, F., Alaiz, M., 2004. Determination of tryptophan by high-performance liquid chromatography of alkaline hydrolysates with spectrophotometric detection. Food Chem. 85, 317–320.