Cloning and Molecular Characterization of LECASAI Lectin Gene from Garlic (Allium Sativum L.)

Neha Gogia¹, Pankaj Kumar², Jitender Singh³, Anchal Rani³, Anil Sirohi⁴ and Prasann Kumar⁵

¹Genetic Engineering of Bacteria laboratory, Division of Bacteriology and Mycology, Indian Veterinary Research Institute Izatnagar, India
²Centre of Excellence in Agriculture Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, U.P., India
³Department of Immunology and Defense Mechanism, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, U.P., India
⁴Department of Molecular Biology & Genetic Engineering, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, U.P., India
⁵Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, 221005, Uttar Pradesh, India.

Email: nehag0609@gmail.com

Abstract

Lectins have always demonstrated their role in bearing insecticidal activity. They are although considered to be toxic for mammals but some studies have reported that the presence of mannose binding region is believed to impart non-toxic nature to lectins against mammals including humans. In the present investigation, the research was conducted to elucidate the relationship of lectin gene LECASAI with other previously cloned lectins having insecticidal activity and to ensure the presence of the conserved mannose binding region/site in LECASAI. In this study, we report isolation of LECASAI via PCR, Cloning, Characterization & Bioinformatics analysis of LECASAI isolated from Garlic (Allium sativum L.). The full length gene sequence of LECASAI obtained from sequencing consisted of 1029bp which was subjected to In-silico analysis in order to determine its ancestral relationship and the presence of the conserved mannose binding region within the sequence. Results from In-vitro analysis indicated a higher homology of LECASAI with those of insecticidal lectins and the presence of mannose binding region in LECASAI. This unique approach has not only helped us in understanding the relationship between these genes but may also enable us to obtain an insecticidal lectin carrying insecticidal activity to several insects pests (for which even Bt proteins have been reported to be ineffective), apart from being non-toxic for man, mammals and birds in the future.

Highlights

• Insect pests cause immense destruction and great losses to many of the important crop plants. Bt proteins have although proved as potential candidates against these pests but there are yet no Bt toxins effective against bugs, hoppers or aphids which feed on phloem sap.

• Scientists have reported lectins to possess insecticidal properties against these insect pests but the toxic nature of lectins to man and animals left the researchers in a dilemma.

• Studies are conducted on LECASAI lectin gene to determine its insecticidal activity and non-toxic nature for man, mammals and birds by elucidating the presence of mannose binding region within the gene sequence.

Keywords: Bacillus thuringiensis, LECASAI, Allium sativum I lectin, Mannose Binding lectins
Introduction

In an approach towards increasing the yield and production of crops, the role of insects pests viz aphids, brown and green planthoppers in causing destruction to crops resulting in heavy loss cannot be neglected. These insects pests not only causes severe physiological damage to the crops but also acting as vectors for transmitting viruses like rice tungro virus, ragged (RRSV) and grassy (RGSV) stunt virus (Hibino, 1989; 1996; Mochida et al., 1979; Saxena and Khan, 1989) to important crops like rice (known as staple food in many countries). Much research has been conducted and is still in process in order to control these insect pests. In the present scenario, the commercial transgenic plants expressing insecticidal toxins are basically based on the ectopical expression of toxins derived from Bacillus thuringiensis (Bt) bacterium. The Bt proteins have shown potential to protect different crops from several insects of lepidopteran and coleopteran class (Barton et al., 1987; Peferoen et al., 1990; Koziel et al., 1993; Fujimoto et al., 1993; Wunn et al., 1996; Nayak et al., 1997) with chewing type of mouth parts. However, there are as yet no Bt toxins to control the insect pests which feed on phloem sap such as bugs, hoppers or aphids (Malone et al., 2008).

Therefore, the search for alternatives to Bt toxins that can be used to achieve resistance against these pests has received a lot of attention now. One of the most promising groups of candidate proteins bearing insecticidal properties against those insect’s pests for which Bt gene also has been found ineffective are plant lectins. Plant Lectins are characterized as “Proteins or Glycoproteins of non-immune origin with one or more binding sites per subunit, which can reversibly bind to the specific sugar segments via hydrogen bonds & Van Der Waals interactions” (Lis and Sharon, 1998). The great potential of plant lectins in having insecticidal activity against a large array of insect species belonging to the Coleoptera, Homoptera, Hemiptera, Diptera and Lepidoptera order (Gatehouse et al., 1995; Schuler et al., 1998; Carlini and Grossi-de-Sa, 2002) is well known. But inspite of their noble role in imparting plant resistance against these insect pests, the toxic effects of some lectins and their antinutritional properties in man and animals, has the left the researchers in a dilemma to use plant lectins in further research or not. In this regard, the researchers conducted many experiments, the results of which demonstrated that apart from being insecticidal, many plant lectins posses cytotoxic, fungitoxic, anti-insect and anti-nematode properties which are either in-vitro or in-vivo toxic for higher animals (Oliveira et al., 1994; Peumans and Van Damme, 1995; Oka et al., 1997; Ripoll et al., 2003) including humans but, in some studies on experimental animals (especially) fed on diets containing plant lectins, the evident symptoms found were loss of appetite, decreased body weight and finally death (Liener et al., 1986; Duranti and Gius, 1997; Lajolo and Genovese, 2002).

In this regard, the presence of a conserved mannose binding region in plant lectins belonging to (Mannose binding lectins) MBL superfamilies is a ray of hope as it is believed that the Mannose binding lectins (MMBLs) although bearing insecticidal activities are considered to non-toxic for mammals and birds (Ripoll et al., 2003).

In the present investigation, results from in-silico analysis, reported that LECASAI gene found in genome of garlic (Allium sativum L.), shares homology with some other previously cloned lectin genes bearing insecticidal activity. The results of above findings also reported that the gene was found to carry a conserved mannose binding region (the detailed information is indicated in results section) which allow these lectins to be categorised into a superfamily of mannose-binding monocot lectins (Van Damme et al., 1994) and may add advantage to increase use of lectins in researches.

Thus, keeping all these things in mind, the research was conducted with an aim to isolate, characterize this gene at molecular level, conduct in-silico analysis to know this gene more and to establish the phylogenetic relationship between LECASAI and other cloned lectin genes possessing the similar characteristics features and to investigate the presence of mannose binding region imparting non-toxic nature to lectins against mammals and birds.

Materials and Methods

Collection, preparation of sample from Allium sativum

The bulbs of garlic (Allium sativum), were purchased from a local store during the month of November 2011. The samples were kept aseptically, rinsed with sterile (distilled) water and were allowed to dry to remove the moisture. The samples were then packed into air tight poly-bags and frozed immediately in deep freezer (-20°C) to keep them safely until processing for their use in further DNA isolation steps.
Primer designing for isolation of lectin gene from Allium sativum L. via PCR

The LECASAI sequence was retrieved from NCBI, U.S.A database and the gene specific primers were designed by Fast-PCR bioinformatics software. The primers compatible in Tm, Size and GC content were selected and the sequence analysis for PCR was conducted through Bioedit, BTI software. The primer sequence was sent for synthesis at Bioserve. The forward and reverse primers obtained from Fast-PCR, were of 22bp and 20bp respectively.

Isolation of Lectin gene from Genomic DNA of Garlic

The Genomic DNA isolation was conducted through CTAB method of DNA isolation (standardized in lab), the pellet obtained was dissolved in 50µl of Autoclaved distilled water, which after purification and Quantification was kept at -20°C for further use. DNA amplification was carried out in a 50 µl of reaction mixture containing 10X PCR buffer, 2.5 mM MgCl₂, 10 mM of each dNTP, 1U Taq DNA polymerase, 100-1000ng genomic DNA and 100ng/µl of each of two appropriate primers mixtures in a reaction. The reaction comprises 35 cycles with the following conditions - Denaturation at 94°C for 10 min, Denaturation at 94°C for 30 sec, Annealing at 58°C for 45 sec, Primer extension at 72 °C for 1 min and Final extension at 72°C for 10 min. The primers for the coding regions of LECASAI were Forward primer-"LEC1F", Sequence 5'-ACTACTTCA TCTCCTAAACTAA-3', Reverse primer - "LEC R", Sequence 5'- ACCAGCAAACGGTGACTTA-3'.

Competent cell preparation, ligation and Transformation.

PCR amplified product was eluted from agarose gel using gel extraction kit (Qiagen Inc, USA). Eluted DNA fragment was ligated to pTZ57RT cloning vector (size 2.8kb) at 4°C with overnight incubation. The recombinant construct was transformed to E. coli DH5α competent cells. Transformants were plated on LB agar supplemented with X-gal/IPTG and ampicillin. Recombinant clones were screened based on blue/white colour colony selection. The presence of correct insert in the recombinant clone was done by isolation of Recombinant Plasmid DNA isolation followed by confirmation by Colony PCR and Restriction enzymes digestion with release of 1029 bp insert by digestion of plasmid with EcoRI and Hind III (Fig 3.6). Restriction analysis of the recombinant clone in this case yielded correct restriction pattern. The recombinant clone was sent for sequencing at Bioserve. The nucleotide sequence obtained was submitted to GenBank, NCBI where it was assigned with the accession number JX561228. The sequence was then blast searched to elucidate the sequence homologues in order to determine the ancestral relationship of the gene with other lectin genes and to investigate its nature in the presence of insecticidal activity. The prediction of ORFs, size and positions, nucleotide translation was performed in order to calculate the theoretical pl and molecular mass of LECASAI sequence, and the presence of mannose binding region in the gene of interest.

Results and Discussion

In-Silico analysis of LECASAI sequence

The LECASAI gene sequence was retrieved from NCBI sequence database, USA and was BLAST searched. The results indicated that the gene shares Maximum percent identity with Allium sativum LECASAI and other lectins belonging to various Allium species (Table 1).

| Accession nos | % Identity | Accession nos | % Identity |
|---------------|------------|---------------|------------|
| M85176.1      | 95%        | EU252577.1    | 91%        |
| M85174.1      | 94%        | EU083313.1    | 91%        |
| M85177.1      | 93%        | DQ640308.1    | 91%        |
| M85175.1      | 92%        | DQ083542.1    | 91%        |
| M85172.1      | 95%        | EU083312.1    | 91%        |
| M85171.1      | 92%        | AY866499.1    | 91%        |

Table 1: Blast results indicating the % identity of LECASAI with other lectin genes.
Isolation of LECASAI from Garlic
The two sets of primers "Lec1F and LecR", were designed, synthesized and used to amplify the gene of interest. According to the results obtained, the primers were found to be compatible (Table 2) to amplify the 1029bp of band thereby spanning the region of the gene of interest (Fig 1).

Table 2. Results of PCR amplification obtained from specific gene Primer set.

| S.No. | Primers   | Expected size | Observed Size |
|-------|-----------|---------------|---------------|
| (1)   | Lec1F, LecR | 1029bp        | +             |

Cloning of PCR amplified product into cloning vector
The PCR amplified and eluted band of 1029bp was then ligated into pTZ57R/T cloning vector of size 2.8kb and were then transformed into E. coli DH5α cells. The presence of the cloned fragment was interpreted by blue and white screening technique. The white colonies obtained indicated the positive results while the blue colonies indicated false positives. The plasmid DNA (total size of 3.8kb) was isolated (Fig 2) from the white colonies which gave an idea of the presence of the cloned gene.

Confirmation of gene cloned into cloning vector
The colonies were subjected to colony PCR and were used as a template using the same set of primers in a 50ìl colony PCR reaction. The amplicons of 1029bp size, confirmed the presence of the gene of interest in the recombinant vector (Fig 3).

Restriction of plasmid with EcoRI (Escherichia coli) and Hind III (Haemophilus Influenzae) yielded two fragments of 3.8kb (total size of plasmid) and 1029bp (insert size) with the generation of sticky ends at both the sites (Fig 4). The recombinant clones confirmed with the presence of gene of interest was sent for sequencing at Bioserve company.
Cloning and Molecular Characterization of *LECASAI* Lectin Gene from Garlic (*Allium Sativum* L.)

Fig. 4: Results obtained from Restriction digestion (from left to right: M-Marker; 1,3,5 plasmids from positive clones; 2,4,6 plasmid vector and cloned gene of interest).

Fig. 5: *In-silico* analysis - the information about the size and position of different ORF's present in the *LECASAI* query sequence.

Fig. 6: *In-silico* analysis: Phylogeny of *LECASAI* query sequence (Genbank accession number: JX561228) with other lectins. The phylogenetic tree is based on the nucleotide sequences of previously cloned lectin genes, obtained from BLAST results at NCBI.
In-silico analysis of sequences

Bioinformatics analysis of 1029bp query sequence was conducted. The results obtained are indicated below. The full length nucleotide sequence of LECASAI obtained was Blast, Tblastx searched for the generation of sequence identity matrix and phylogenetic tree construction to see the sequence homology of LECASAI with other lectins in order to explore the presence of the insecticidal activity (Fig 6) and non toxic activity, indicating the presence of mannose binding region (Fig 10) within the query sequence. The query sequence was also analyzed in detail with respect to its ORF’s (Fig 5), putative start/stop codon, pI value, Mwt, Multiple sequence alignment (Fig 9), Interproscan (Fig 8) and was later subjected to search the conserved domains (Fig 7) to know the gene more and to detect and get an idea/positions of the presence of the conserved mannose binding region in the query sequence.

The molecular weight of the full length LECASAI Allium sativum lectin gene sequence was found to be 84395.06 Da. The pI (Isoelectric point value) of LECASAI gene query sequence obtained was 5.07. The pI and Molecular weight was calculated using the Expasy Bioinformatics resource portal. The sequence was later used to obtain ORFs. Six ORFs were found on the LECASAI query sequence, out of them the ORF (frame +1) was found to be the largest ORF among all other ORF which starts with a start codon ATG from 22 position and ends with a stop codon TAG at 912 on plus strand, the total length of ORF comprise is of 891 bp.

The Multiple sequence alignment was done to detect and compare the conserved mannose binding region of the query sequence with that in other MBLs. The Results clearly characterized the query sequence to be a Bulb-type mannose-specific lectin, the domain of which consists of 3-fold internal repeat (beta-prism architecture). The

Fig. 7: In-silico analysis- Conserved mannose binding domains, multi-domains, region and their occurrence within the LECASAI sequence.

Fig. 8: In-silico analysis - Interproscan results showing the matched amino-acid sequences of mannose binding regions in LECASAI sequence with other Allium species seaqences (submitted in a total of 14 databases).
Cloning and Molecular Characterization of LECASAI Lectin Gene from Garlic (Allium Sativum L.)

Fig. 9: In-silico analysis - Multiple sequence alignment (MCS) indicating the presence of consensus sequence motif QXDXXVXY responsible for detection of alpha-D-mannose binding region.

presence of consensus sequence motif QXDXXVXY was found to be involved in the recognition of alpha-D-mannose binding region. The full length LECASAI gene sequence was then used to locate the positions of conserved mannose binding domains via Conserved Domain Search Service (CD Search) tool at NCBI. According to the results obtained the mannose binding region was found to be present in a range of 150-375bp and 600-825 bp within the query sequence. The triangles represent the amino acids comprising conserved regions/sites mapped from conserved domain annotations. The specific hits/ multi domains/B-lectin superfamilies region comprise a region between 75-400bp and 525-850 bp while the dimerization interface was occupied from 75-400bp and 525-850bp. However, the low complexity region was found between 25-75 base pairs. Thus, the query sequence was confirmed to belong to the super family of Mannose binding Lectins.

The phylogenetic tree of the Alliaceae lectins reveals some interesting evolutionary relationships. According to the results obtained in dendrogram tree analysis lectins were divided into three major groups. One group contained lectins viz. ASAI, LECASAI all derived from garlic (A. sativum). It highlighted and confirmed that the query sequence was grouped together with LECASAI and ASA genes found in garlic (from which the query sequence was actually obtained). The second group comprises of lectins...
from leaf, rhizome, seed and tuber (with mannose binding feature in common). The third group purely contained mannose specific lectins derived from *A. cepa* species. Out of these lectins most of the lectins broadly have already been reported to belong to the superfamilies of mannose binding lectins and some with being insecticidal too. Thus, it can be indicated that all these groups of lectins were derived from a common ancestor during evolution, suggesting that these lectins shared a common evolutionary ancestral relationship. A similar result were observed in evolutionary relationship of lectin gene with deduced amino acid sequences for the presence of mannose binding regions which showed almost similar results as mentioned above and confirmed that the *A. sativum* query sequence is highly related to *LECASAI* & *ASAII* (all derived from *A. sativum*), followed by lectins present in different plant parts of *A. sativum* or other Allium species including *Allium cepa*.

This study, *LECASAI* bulb specific gene which refers to *Allium sativum* agglutinin I (ASAI) - a heterodimeric bulb lectin composed of 11.5 kDa subunit (Smeets et al., 1997) which through bioinformatics analysis was found to be homologous to lectins bearing insecticidal activity and also to those lectin genes carrying the conserved mannose binding region. The bulb was found to be a bulb Mannose binding Lectin (MBL) and the plant lectin gene *LECASAI* was predicted to carry insecticidal activity by being non toxic for mammals.

The results from BLAST showed a high degree of similarity of *LECASAI* with other lectins present in different Allium species carrying insecticidal activity. The results showed that *LECASAI* gene shares a percent homology of 95%, 94%, 93%, 92%, 91% with published *LECASAI3,ASAII2* (Genbank Accession no: M85176,M85172), *LECASAI1* (M85174), *LECASAI4* (M85177), *LECASAI2,ASAII1* (all derived from *A. sativum*)
The region (translated protein) in query sequence specific for coding bulb-type mannose-specific lectin containing a consensus sequence motif QXDXNXVXY involved in alpha-D-mannose recognition was scanned for InterProScan results (the software allows us to scan the sequence for matches against the InterPro collection of 14 protein signature databases), in order to confirm the presence of lectin domain/mannose binding region in the query sequence. The results found were satisfactory, clearly indicating and confirming the presence of bulb-type lectin domain region within the query sequence. Interestingly, the results from tblastx and the phylogenetic tree analysis of LECASAI gene (for confirmation of the presence of conserved mannose binding region) were also found exactly same as that of BLAST results. The query sequence also showed a higher degree of homology with respect to mannose binding regions with other MBLs too.

**Conclusion**

Thus, taking into consideration the enigmatic nature of lectins in sharing similar overall characteristics features despite varying identities and the potential of plant lectins in providing plants resistance against various insect pests, the search for more lectin genes has became a necessity. It has been found that the mannose binding garlic lectins are closely related proteins sharing conserved mannose binding regions (a feature of most mannose binding lectins), found to be encoded by homologues of LECASAI (M85175.M85171) and ASAII3 (M85173). A relatively less percent homology of 88% was identified with Allium cepa lectin (DQ255944).

The region (translated protein) in query sequence specific for coding bulb-type mannose-specific lectin containing a consensus sequence motif QXDXNXVXY involved in alpha-D-mannose recognition was scanned for InterProScan results (the software allows us to scan the sequence for matches against the InterPro collection of 14 protein signature databases), in order to confirm the presence of lectin domain/mannose binding region in the query sequence. The results found were satisfactory, clearly indicating and confirming the presence of bulb-type lectin domain region within the query sequence. Interestingly, the results from tblastx and the phylogenetic tree analysis of LECASAI gene (for confirmation of the presence of conserved mannose binding region) were also found exactly same as that of BLAST results. The query sequence also showed a higher degree of homology with respect to mannose binding regions with other MBLs too.

**Accession number**

The sequence of LECASAI gene was submitted to GenBank via Bankit where it was assigned the accession number JX561228 by National Center for Biotechnology Information (NCBI), U.S.A.

**Acknowledgements**

The Author is thankful to the Council of Science and Technology, (U.P-India), Department of Biochemistry and Physiology, Immunology and Defense Mechanism, Sardar Vallabhbhai Patel university of Agriculture and Technology, Meerut for providing facilities to undergo the study. The help of Miss. Rosy Rani, Miss. Juhi Bhardwaj, Mr. Amit Kumar (SRFs) and Mr. Harish Chandra is sincerely acknowledged.

**References**

Bandyopadhyay, S., Roy, A., and Das, S. 2001. Binding of garlic (*Allium sativum*) leaf lectin to the gut receptors of homopteran pests is correlated to its insecticidal activity. *Plant Science* **161**: 1025-1033.

Barton, K., Whiteley, H., and Yang, N.S. 1987. *Bacillus thuringiensis* endotoxin in Transgenic *Nicotiana tabacum* provides resistance to lepidopteran insects. *Plant Physiology* **85**: 1103-1109.

Carlini, C.R., and Grossi-de-Sa, M.F. 2002. Plant toxic proteins with insecticidal properties. A review on their potentialities as bioinsecticides. *Toxicon* **20**: 1515-1539.

Chandra, N.R., Ramachandraiah, G., Bachhawat, K., Dam, T.K., Surolia, A., and Vijayan, M. 1999. Crystal structure of a dimeric mannose specific agglutinin from garlic: quaternary association and carbohydrate specificity. *Journal of Molecular Biology* **285**: 1157-1168.

De Kochko, A., and Hamon, S. 1990. A rapid and efficient method for isolation of restrictable DNA from plants of genus *Abelmoschus*. *Plant Molecular Biology Reporter* **8**: 3-7.

Dellaporta, S.L., Wood, J., and Hicks, J.B. 1983. A plant DNA mini-preparation: version II. *Plant Molecular Biology Reporter* **1**: 19-21.

Down, R.E., Gatehouse, A.M.R., Hamilton, W.D.O., and Gatehouse, J.A. 1996. Snowdrop lectin inhibits development and decreases fecundity of glasshouse potato aphid (*Aulacorthum solani*) when administered *in-vitro* and *via* transgenic plants in laboratory and glasshouse trials. *Journal of Insect Physiology* **42**: 1035-1045.

Duranti, M., and Gius, C. 1997. Legume seeds: protein content and nutritional value. *Field Crops Research* **53**: 31-45.

Fujimoto, H., Itoh, K., Yamamoto, M., Kyozuka, J., and Shimamoto, K. 1993. Insect resistant rice generated by introduction of a modified endotoxin gene of *Bacillus thuringiensis*. *Biotechnology* **11**: 1151-1155.

Gatehouse, A.M.R., Powell, K.S., Van Damme, E.J.M., Peumans, W.J., and Gatehouse, J.A. 1995. Insecticidal properties of plant lectins: their potential in plant protection. In: Pustzai, A., Bardocz, S. (Eds.), Lectins: Biomedical Perspectives: 35-58.

Hibino, H. 1989. Insect borne viruses in rice. *Springer Verlag* **6**: 209-241.

Hibino, H. 1996. Biology and epidemiology of rice viruses. *Annual
Review of Phytopathology 34: 249-274.

Kozak, M. 1981. Possible role of flanking nucleotides in recognition of the AUG initiation codon by eukaryotic ribosomes. *Nucleic Acids Research* 9 (20): 5233-5252.

Koziel, M.G., Beland, G.L., Bowman, C., Carozzi, N.B., Crenshaw, R., Crossland, L., Dawson, J., Desai, N., Hill, M., and Kadwell, S. 1993. Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*, *Biotechnology* 11: 194-200.

Lajolo, F.M., and Genovese, M.I. 2002. Nutritional significance of lectins and enzyme inhibitors from legumes. *Journal of Agricultural and Food Chemistry* 50: 6592-6598.

Liener, I.E., and Sharon, N. 1998. Lectins: carbohydrate specific proteins that mediate cellular recognition. *Chemical Reviews* 98: 637-674.

Malone, L.A., Gatehouse, A.M.R., and Barratt, B.I.P. 2008. Beyond Bt: alternative strategies for insect resistant genetically modified crops. In: Romeis, J., Shelton, A.M., Kennedy, G.G. (Eds.), Integration of Insect-Resistant Genetically Modified Crops within IPM Programs. *Springer*, Dordrecht, pp. 357-417.

Mierendorf, R.C., and Pfeffer, D. 1987. Direct sequencing of denatured plasmid DNA. *Methods in Enzymology* 152: 556-562.

Mochida, O., Wahyu, A., and Surjani, T.K. 1979. Some Considerations on Screening Resistant Cultivars/Lines of Rice Plant to the Brown Planthopper, *Nilparvata lugens* (Stal) (Hom., Delphacidae). Los Banos, Philippines: IRRI, pp. 1-9.

Nayak, P., Basu, S., Das, S., Basu, A., Ghosh, D., Ramakrishnan, N.A., Ghosh, M., and Sen, S.K. 1997. Transgenic elite indica rice plants expressing Cry1Ac endotoxin of *Bacillus thuringiensis* are resistant against yellow stem borer (*Scirpophaga incertulas*). *Proceedings of the National Academy of Sciences* 94: 2111-2116.

Oka, Y., Chet, I., and Spiegel, Y. 1997. Accumulation of lectins in cereal roots invaded by the cereal cyst nematode *Heterodera avenae*. *Physiological and Molecular Plant Pathology* 51: 333-345.

Oliveira, J.T.A., Vasconcelos, I.M., Gondim, M.J.L., Cavada, B.S., Moreira, R.A., Santos, C.F., and Moreira, L.L.M. 1994. *Canavalia brasiliensis* seeds. Protein quality and nutritional implications of dietary lectin. *Journal of the Science of Food Agriculture* 64: 417-424.

Pfeiroen, M., Janssen, S., Reynaerts, A., and Leemans, J. 1990. Potato plants with engineered resistance against insect attack. In: Molecular and cellular biology of the potato (eds) M Vayda and W Park (Tucson: CAB) pp 193-204.

Peumans, W.J., and Van Damme, J.M. 1995. Lectins as plant defense proteins. *Plant Physiology* 109: 347-352.

Powell, K.S., Gatehouse, A.M.R., Hilder, V.A., Van Damme, E.J.M., Peumans, W.J., Boonjawat, J., Horsham, K., and Gatehouse, J.A. 1995. Different antimetabolic effects of related lectins towards nymphal stages of *Nilaparvata lugens*. *Entomologia experimentalis et applicata* 75: 61-65.

Ripoll, C., Favery, B., Lecomte, P., Van Damme, E., Peumans, W., Abad, P., and Jouanin, L. 2003. Evaluation of the ability of lectin from snowdrop (*Galanthus nivalis*) to protect plants against root-knot nematodes. *Plant Science* 164: 517-523.

Saxena, R.C., and Khan, Z.R. 1989. Factors affecting resistance of rice varieties to planthopper and leafflower pests. *Agricultural Zoology Reviews* 3: 97-132.

Schuler, T.H., Poppy, G.M., Kerry, B.R., and Denholm, I. 1998. Insect resistant transgenic plants. *Trends in Biotechnology* 16: 168-174.

Smeets, K., Van Damme, E.J.M., Verhaert, P., Barre, A., Rouge, P., Van Leuven, F.V., and Peumans, W.J. 1997. Isolation, characterisation and molecular cloning of the mannos binding lectins from leaves and roots of garlic (*Allium sativum* L.). *Plant Molecular Biology* 33: 223-234.

Van Damme, E.J.M., and Peumans, W.J. 1993. The mannose binding lectins from *Alliaceae* Species. *Lectins Biology-Biochemistry, Clinical Biochemistry* 9: 9-18.

Van Damme, E.J.M., Goldstein, I.J., and Peumans, W.J. 1991a. A comparative study of related mannose binding lectins from the *Amaryllidaceae* and *Alliaceae*. *Phytochemistry* 30: 509-514.

Van Damme, E.J.M., Smeets, K., and Peumans, W.J. 1994. Molecular cloning of the mannos binding lectins from *Amaryllidaceae* and *Alliaceae* species. *Lectin Biology, Biochemistry and Clinical Biochemistry* 9: 166-177.

Van Damme, E.J.M., Smeets, K., Torrekens, S., Leuven, F.V., Goldstein, I.J., and Peumans, W.J. 1992. The closely related homomeric and heteromeric mannose binding lectins from garlic are encoded by one domain and two domain lectin genes. *European Journal of Biochemistry* 206: 413-420.

Wunn, J., Kloti, A., Burkhardt, P.K., Biswas, G.C., Ghosh Launis, K., Iglesias, V.A., and Potrykus, I. 1996. Transgenic indica rice breeding line IR85 expressing a synthetic Cry1A (b) gene from *Bacillus thuringiensis* provides effective insect pest control. *Biotechnology* 14: 171-176.