INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
13–778/2015/17–3–547–553
DOI: 10.17957/IJAB/17.3.13.778
http://www.fspublishers.org

Full Length Article

Computational and Real-Time RT-PCR Expression Analyses of Garlic Lectin Gene

Faiqa Usman, Sameera Rehman, Muhammad Ramzan Khan* and Ghulam Muhammad Ali*
National Institute for Genomics and Advanced Biotechnology, National Agricultural Research Center, Park Road, Islamabad, Pakistan
*For correspondence: drmrkhan_nigab@yahoo.com; drgmali@yahoo.ca

Abstract

Sucking insect pests inflict heavy losses to important crops. Bt gene being unable to control sucking insects, *lectin* seems to be a good alternative insecticidal gene. However, studies on sequence analysis, evolutionary relationship and expression patterns of garlic *lectin* genes remained elusive. Therefore, this study was envisaged to explore *lectin* gene sequence diversity in different species, and to analyze differential expression of *lectin* genes in garlic bulb, leaf and root tissues. Phylogenetic reconstruction did not reveal well-defined clusters indicating divergence in *lectin* sequences in different plant species. Multiple alignments exhibited conservation in *lectin* coding sequences in the middle regions. On the other hand, both the N- and C-termini were quite divergent and size of the coding regions varied greatly in different orthologs probably due to exonization of introns. Semi-quantitative and quantitative RT-PCR exhibited elevated expression of *lectin* genes in garlic bulb as compared to leaf and root tissues. this study has future implications in transforming insecticidal genes in crops against sucking insects.

Keywords: Lectin; Garlic; Gene; Insect resistance; Real-time RT-PCR; Expression

Introduction

Sucking insect pests effectuate great losses to important crops in Pakistan including oil seed crops such as mustard, rapeseed, wheat, rice and cotton (Salman *et al*., 2011). Chemical pesticides bring about negative environmental and health effects (Ramzan *et al*., 2008). Bt gene cannot control sucking insects such as white fly, thrips, and jassids (Hussain *et al*., 2008). In contrast, important insect resistant proteins such as lectins are carbohydrate binding storage proteins distributed widely in the plant kingdom. Insecticidal properties of lectins have been reported in a number of studies against various insects such as European corn borer (*Ostrinia nubilalis*), homopeterans, leidopterans and coloeopterans. Studies have shown that lectins affect survival and development of insects (Powell *et al*., 1995). Lectins attach to the glycosylated receptors present on the lining of insect's gut. These also interfere with development of peritrophic membrane of insect's midgut. Lectins can recognize specific carbohydrate moieties thereby controlling various cellular functions as serum glycoprotein turnover, cell-to-cell, host and pathogen interactions and a number of innate immune responses (Ehsan and Altaf, 2009). More than 60% reduction in pest population can be observed after treating with soybean lectin, chickpea lectin and garlic lectin.

Garlic lectins are included in the class of GNA (*Galanthus nivalis*) like lectins. Garlic (*Allium sativum*) is a monocot and belongs to Alliaceae family. These lectins have greater diversity in their carbohydrate binding and molecular structure. Initially it was reported that lectins can only bind to mannose but studies have shown that lectins have wide range of carbohydrate specificities. There are two main classes of lectins; constitutive lectins and inducible lectins. When wheat is infested with Hessain fly (*Mayetiola destructor),* it triggers the expression of jacalin-like lectin. Examples of constitutive lectin include garlic lectins, *Amaranthus caudatus* galactose specific lectin, jackbean lectin from Concanavalin A, and Ricin B lectins (Sauvion *et al*., 2004; Michiels *et al*., 2010).

Previous studies have only focused on the glycosylation patterns and protein structural analysis of lectins (Pilobello *et al*., 2005; Chakraborti *et al*., 2009), lectin protein expression and insecticidal efficacy based studies *via* expression in *Pichia pastoris* (Aslam and Gilani, 2000), and statistical study of multivalent nature of lectins (Damme *et al*., 1992). Transformation studies have also been conducted to study efficacy of lectins against sucking insects in pyramided rice lines (Bharathi *et al*., 2011), marker gene-free transgenic rice (Yarasi *et al*., 2008; Sengupta *et al*., 2010), tobacco (Sadeghi *et al*., 2008) and chickpea (Chakraborti *et al*., 2009). However, studies have not been conducted on the gene expression levels of *lectin* and roles of its protein structure in insecticidal activity. Hence, before *lectin* genes are used for transformation

To cite this paper: Usman, F., S. Rehman, M.R. Khan and G.M. Ali, 2015. Computational and real-time expression analyses of lectin gene. *Int. J. Agric. Biol*., 17: 547–553

studies, focus should be given on its expression and sequence analysis that can help in defining the structural regions that play roles in insecticidal activity of *lectins*. Therefore, sequence conservation of *lectin* genes need to be studied for an in-depth learning of its roles in insect resistance. Furthermore, elucidation of expression levels of *lectin* genes in different tissues of the plants during different developmental stages remained fragmented. Therefore, this study was devised to understand the relative expression levels of *lectin* genes in selected tissues of garlic (*A. sativum*) with real-time PCR. Phylogenetic analysis based on coding sequence of *lectin* genes from different plant sources can help in studying evolutionary relationships of the *lectin* genes. Therefore the major objectives of this study were (1) to do computational and phylogenetic analysis of *lectin* gene sequences and (2) to perform expression analysis of *lectin* genes in garlic (*A. sativum*) bulb, leaf and root tissues using real-time RT-PCR.

Based on computational and expression analyses we posit that no well-defined clusters are featured by the *lectin* gene phylogeny in different plants species. There is conservation in the middle domain of the *lectin* coding sequence. Nevertheless, both the N- and C-termini are quite diverse, and due to exonization of introns the size of the orthologs is also variable. The expression is concerted in the bulb tissue in comparison with leaf and root.

Materials and Methods

Plant Materials and Growth Conditions

Plants of local "Hazro" and Chinese "Chinese Purple" varieties of garlic (*A. sativum*) were grown in glasshouse at 25˚C at National Institute for Genomics and Advanced Biotechnology, National Agricultural Research Centre, Islamabad, Pakistan.

Nucleic Acids Extraction and First Strand cDNA Synthesis

Total RNA of garlic leaf, root and bulb was extracted for gene expression analysis using PureLink™ RNA Mini Kit (Invitrogen). Synthesis of first strand cDNA was carried out through Fermentas™ RevertAid™ M-MuLV Reverse Transcriptase (Cat. No. K1621) using Oligo dT primer.

Phylogenetic Reconstruction and Sequence Analysis

For computational and phylogenetic analyses of lectin gene, nucleotide coding sequences from 31 different plant sources were retrieved from NCBI (www.ncbi.nlm.nih.gov) databases (Ramachandraiah *et al*., 2003). In order to infer their evolutionary relationships Maximum Likelihood (ML) algorithm was employed using MEGA5 program (Kumar *et al*., 2008) keeping default settings. Bootstrap values for 1000 replications were calculated for the reliability of the ML tree.

To analyze the diversity of lectin orthologs from various plant species, nucleotide sequence diversity was studied using MacVector™ 7.2.3. (Accelrys Inc.) gcg/Wisconsin Package University of Wisconsin) software. Multiple alignments of translated sequences of lectin genes were generated using ClustalW program in MacVector™ 7.2.3. The conserved and variable regions were identified.

Semi-Quantitative RT-PCR

Multiplex semi-quantitative RT-PCR was performed in a single reaction with total volume of 25 µL using Fermentas™ Taq Polymerase (Catalogue No. EP0402) as described by Khan *et al*. (2012). The forward primer sequence was 5'-GGTCTGTACGCAGGCCAATCCCTA-3' and that of reverse was 5'-CCACAAAGTTGCCATCAGCTGCA-3'. The cycling conditions for *lectin* gene amplifications were 95°C for 2 min to activate Taq polymerase, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing 58°C for 30 min, extension at 72°C 30 min and a final extension at 72°C for 7 min. The *18SrRNA* gene was used as internal control. During this experiment three biological as well as technical replicates were used. PCR products were resolved on 2% agarose gel and photographed.

Quantitative Real-time RT-PCR

Detailed expression of *lectin* gene in three garlic tissues was investigated by quantitative real-time RT-PCR using StepOnePlus™ real-time PCR Systems. The comparative C_T ($\Delta\Delta$Ct) method was adopted for relative expression analysis. Two internal controls were employed. These include *18SrRNA* and *Tubulin* genes. Reactions were carried out in a volume of 20 µL including 300 nmol/L of gene-specific primers and Maxima® SYBR Green/ROX qPCR Master Mix (2X) (Fermentas). The reaction conditions were set at 95°C for 10 min to activate polymerase, followed by 45 cycles of 95°C for 30 sec, 60°C for 30 sec, and a final melting curve analysis from 60°C to 95°C. The reaction was repeated in triplicate for each of the biological as well as technical replicates. Relative expression of garlic *lectin* gene was normalized with respect to *18SrRNA* and *Tubulin* genes as internal controls. Standard deviation was determined. Bar chart was generated in excel sheet.

Results

Phylogenetic and Sequence Analyses of *Lectin* Genes

In order to elucidate the phylogenetic relationship among lectin orthologs from various plant species including *A. cepa*, *A. porrum*, *A. sativum*, *Vigna radiate*, *Pisum sativum*, *Viscum album*, *Hordeum vulgare* and *Arabidopsis thaliana* etc. Maximum Likelihood tree was re-constructed in

Table 1: List of *lectin* homologs from different plant species for phylogenetic reconstruction. Their accession numbers for NCBI GenBank along with length of the coding sequences in base pair have been given. CDS, stands for nucleotide coding sequence

Common Names	Species	GenBank Accession	CDS (bp)
Common mushroom	*Agaricus bisporus*	U14936.1	464
Onion	*Allium cepa*	DQ255944.1	334
Leek	*Allium porrum*	L12173.1	542
Galric	*Allium sativum*	DQ525625.1	547
Thale cress	*Arabidopsis thaliana*	AB638778.1	475
Groundnut	*Arachis hypogaea*	AY431029.1	844
Pigeon pea	*Cajanus cajan*	JN561784.1	828
Coral erythrina	*Erythrina corallodendron*	X52782.1	845
Coral Tree	*Erythrina crista-galli*	AY158072.1	727
Soybean	*Glycine max*	NM_001250281.2	850
Barley	*Hordeum vulgare*	AK356321.1	441
Lentil	*Lens culinaris*	DQ005103.1	829
Castor oil	*Ricinus communis*	S40368.1	1624
Lentil	*Lens ervoides*	AJ318220.2	829
Lima bean	*Phaseolus lunatus*	J05053.1	788
Barrel Clover	*Medicago truncatula*	X60386.1	833
Barrel Clover	*Medicago truncatula*	X60387.1	840
Pea	*Pisum sativum*	M18160.1	827
Lima Bean	*Phaseolus lunatus*	L26237.1	769
Rice	*Oryza sativa*	M24504.1	683
Pea	*Pisum sativum*	U31981.1	812
Pea	*Pisum sativum*	Y00440.1	827
Mukwa	*Pterocarpus angolensis*	AJ426056.1	784
Pagoda Tree	*Sophora japonica*	U63011.1	879
Pagoda Tree	*Sophora japonica*	AJ234397.1	315
Sea lettuces	*Ulva pertusa*	AY433960.1	611
European Mistletoe	*Viscum album*	AY377890.1	1694
Cowpea	*Vigna unguiculata*	AM494005.1	467
Mung bean	*Vigna radiata*	HM348715.1	827
Moth bean	*Vigna aconitifolia*	JF501650.1	844
Broad bean	*Vicia faba*	AJ438593.2	832

MEGA5 (Table 1). Fig. 1 demonstrates that there is no clear differentiation into different clades. Some of the sequences (*Arabidopsis*, *Hordeum*, *Ulva* etc.) are scattered as individual, while other are so closely related as they are sisters even though they belong to different species (*Lens*, *Cajanus* and *Vigna* etc.). This depicts that lectin gene in different species has not diverged in a sequential manner. *Allium* species seem to the progenitors. However, no specific lineages are observed. The *Allium* species are clustered together showing their sister relationship. These are also located at the basal position. Bootstrap values obtained are higher in the ML trees that are indicative of increased reliability of the tree. Sequence conservation can only be observed in *lectin* genes from plants of same species as *Allium* sp. This reflects great divergence in the *lectin* during the course of evolution. Nonetheless, no clustering was observed in *lectin* genes from different species. This allows inferring that sequences of these genes display polymorphism. The observed divergence of the *lectin* genes was investigated and conserved regions were focused.

First of all, coding sequences of *lectin* genes were aligned using ClustalW program in MacVector™. Great variations in the length of the coding sequences of *lectin* gene were observed. *Allium* species has 350bp coding sequence, while length of the coding sequence in *Pisum sativum* is 827 bp. Surprisingly, *Viscum album* contains a very long 1694bp coding sequence. Multiple alignments highlighted two different groups (Fig. 2). *Allium* and *Hordeum* species exhibited conserved clustered in different patterns than the rest of the sequences. Overall, patches of homology were observed in the middle of the sequences. Nevertheless, no clear cut conserved regions were detectable in the alignment. The alignment of amino acid sequences showed regions with conservation that could be involved in lectin's insecticidal role. The principal of phylogenetic foot printing is that the conserved regions are likely to be functionally active. In the overall alignment, no highly conserved regions were observed. Therefore, alignments on smaller scale were focused. For this purpose, only *Allium* lectins were selected. Fig. 3 demonstrates that within these species, lectin gene is highly conserved. What are the domains or regions specific for insecticidal activity can be traced through site-directed mutagenesis and functional characterization. *Lectin* genes seem to be very important in conferring resistance to plants against insects, hitherto little efforts have been made to reveal detailed expression patterns of these genes using modern PCR based expression techniques such as real time PCR. Therefore in order to validate and elaborate the expression patterns, real time PCR was employed.

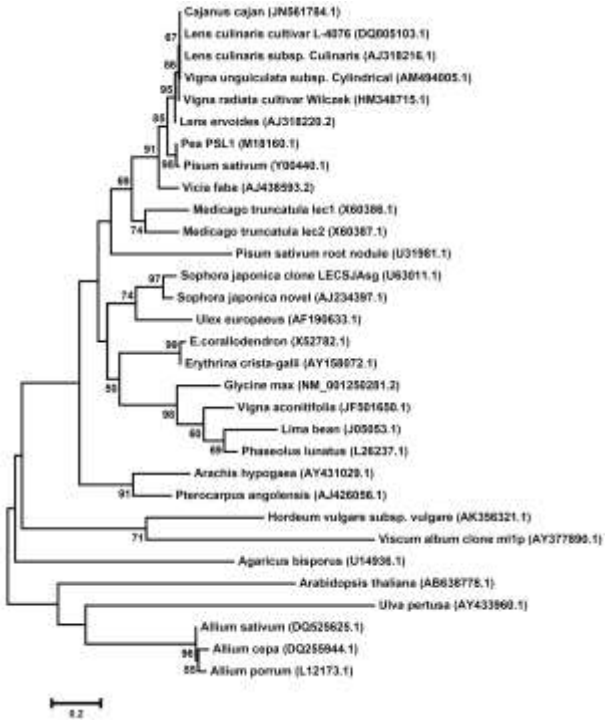

Fig. 1: Phylogenetic reconstruction of *lectin* gene from different plants species. Tree was constructed using Maximum Likelihood algorithm in MEGA5. Numbers indicate the bootstrap calculations for 1000 replicates

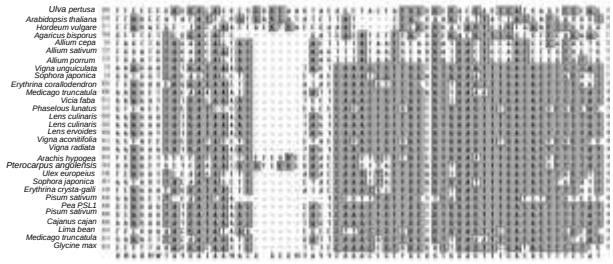

Fig. 2: Partial multiple alignments for *lectin* homologs from various plant species. ClustalW multiple alignment was performed with MacVector™ 7.2.3. (Accelrys Inc.) gcg/Wisconsin Package University Of Wisconsin). The nucleotides in blocks highlight conserved regions. Consensus is also shown at the bottom

Lectin is Differentially Expressed in Garlic Tissues

In order to discern the expression patterns of *lectin* gene, semi-quantitative RT-PCR analysis was employed with gene specific primers for the coding region of the *lectin* gene using *18SrRNA* as an internal control. For this purpose, total RNA of leave, root and bulb tissues of garlic was isolated and converted into cDNA (Table 2). Fig. 4 shows that the transcript signals of *lectin* are strongly detectable in the bulb tissues in comparison with leaf and root.

Table 2: Varieties and tissues used for extraction of total RNA for *lectin* gene expression analysis

Sr. No.	Garlic Variety	Organ	Tissue
1	Hazro	Bulb	Mature Bulb
			Immature (Young) Bulb
		Leaf	Meristematic Tip
			Mature Leaf
		Roots	Primary Root
			Secondary Root
			Tertiary Root
2	Chinese Purple	Bulb	Mature Bulb
			Immature (Young) Bulb
		Leaf	Meristematic Tip
			Mature Leaf
		Roots	Primary Root
			Secondary Root
			Tertiary Root

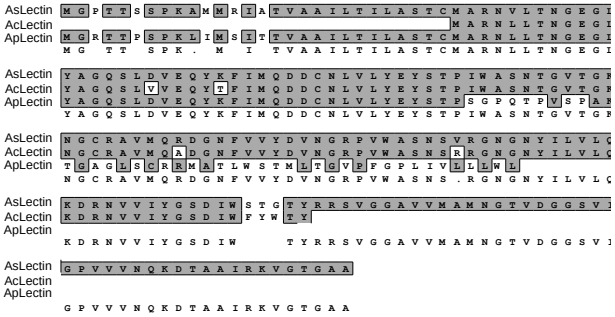

Fig. 3: Multiple alignments for *Lectin* homologs from *Allium* plant species. ClustalW multiple alignment of *lectin* amino-acids was performed with MacVector™ 7.2.3. (Accelrys Inc.) gcg/Wisconsin Package University Of Wisconsin). The amino acids in blocks highlight conserved regions. Consensus is also shown at the bottom. AsLectin symbolizes the *A. sativum* Lectin; AcLectin, *A. cepa* Lectin; ApLectin, *A. porum* Lectin

There is moderate expression in leaf, which becomes weaker in root but still detectable. Variations in expression in different tissues are also quite prominent for both the local (Hazro) and Chinese varieties (Chinese Purple). Remarkably, in both the species, gene is expressed at the same level. What might be the reason behind the stronger expression of *lectin* gene in bulb tissue? It is speculated that being a storage organ, this tissue exhibits the stronger expression that might enable this gene to combat against insect pests. However, these speculations require empirical evidences.

Real time RT-PCR of *lectin* genes from different tissues of both the local and Chinese varieties was performed using Applied Biosystems StepOnePlus™ Real-Time PCR Systems. It is evident from Fig. 5 that like semi-quantitative RT-PCR, real time PCR also exhibits very strong signals in bulb tissues. In leaf tissues, the signals are detectable that become weaker in root tissues. No differences in varietal expression patterns are observed.

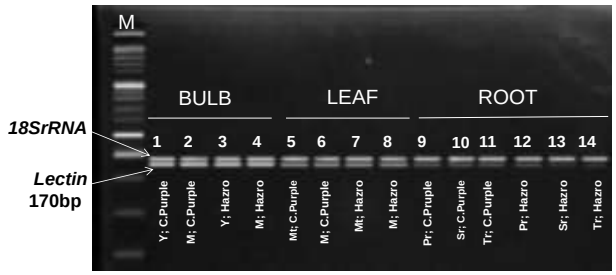

Fig. 4: Gene expression analysis of garlic *lectin* genes in garlic bulb, leaf and root tissues. Expression analysis of *lectin* genes in three tissues of garlic; bulbs, roots and leaves carried out using semi-quantitative RT-PCR. Relative expression was normalized with respect to housekeeping gene *18SrRNA*. M, 50bp ladder as marker; Y, Young; C, Chinese; M, Mature; Pr, Primary root; Sr, Secondary root; Tr, Tertiary roots

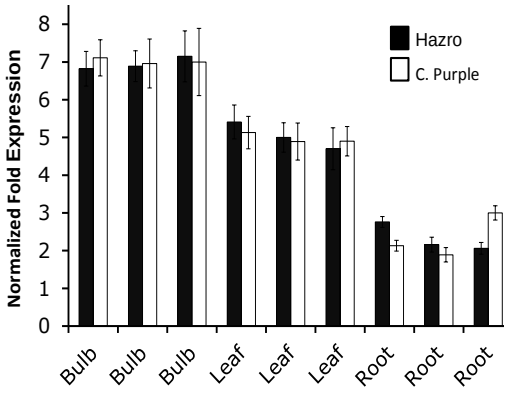

Fig. 5: Garlic lectin gene expression analysis using real time RT-PCR. The comparative C_T ($\Delta\Delta$Ct) method was adopted for relative expression analysis. The RNAs isolated from bulb, leaves and root of garlic were subjected to real-time RT-PCR analysis with gene specific primer pairs. The columns show the expression of Hazro (Balck) and Chines Purple (White). The values given are relative expression based on three independent experiments normalized with respect to 18SrRNA and Tubulin. Error bars indicate the standard deviation of the mean

The genes in both the varieties are expressed at the same level in different target tissues.

Cumulative data from sequence divergence and real time RT-PCR suggests that *lectin* genes have variable length in different species and these genes are not clearly differentiated into different lineages. Their expression patterns are diverse being stronger in bulb in comparison with leaf and root.

Discussion

Insect pests are one of the major disasters to agricultural crops in Pakistan. Chewing insects (lepidopterans) and sucking insect pests (hemipterans) pose challenge to mustard, rapeseed, wheat, rice and cotton (Salman *et al*., 2011). Genetic manipulation remained an important tool to create environmental friendly strategies to combat insect pest attack on important crops. Insecticidal genes hold importance in opposition to insecticides and pesticides that pose human health adversities and harm to the environment (Ramzan *et al*., 2008). There are two major classes of genes used against these two classes of insects. *Bt* gene, which is effective against chewing insects and *lectin* genes, which are used to combat sucking insect pests. *Bt* gene is specific requiring alkaline environment of target insect gut. Furthermore, insects develop resistance against Bt gene over a period of five years (Tajne *et al*., 2012). Besides, based on its specificity, Bt gene is not effective against sucking insects. Sucking insects including aphids (Hussain *et al*., 2008), jassids, thrips and whitefly damage crops in two ways. Firstly, sucking insects suck plant sap/phloem, thus depriving the plant of nutrition. Secondly, secretion of honeydew by sucking insects on the surface of attacked plant tissue attracts further pathogens as fungi, viruses and bacteria. The spread of cotton leaf curl virus on cotton by whitefly is an important example (Amjad *et al*., 2009). Genetic engineering studies have used many genes to combat insect attack. Protease inhibitors (Haq *et al*., 2004), toxin A produced by *Photorhabdus luminescens* (Liu *et al*., 2003), bacterial cholesterol oxidase (Corbin *et al*., 2001) and avidin from birds are some of the examples. Limitations of these genes lie in their specificities against insects.

In sharp contrast with Bt gene, lectins are carbohydrate specific proteins widespread in the plant kingdom including red kidney beans, wheat, lentils and legumes (Sauvion *et al.*, 2004). Garlic lectins are mannose specific proteins. Lectins take advantage of glycoproteins present on exteriors of epithelial cellular on insect's gut. Lectins recognize specific carbohydrate moiety on the epithelial lining of the insect's intestine. Conformation of glycoproteins on epithelial lining is changed, causing pore formation in the gut surface, leading to leaky gut. This leads to great failures of digestive mechanisms. Conformational changes of gut surface glucoproteins affect cell-to-cell interaction (Ehsan and Altaf, 2009). Garlic *lectins* remained the focus of various transformation studies. Studies such as pyramided rice lines (Foissac *et al*., 2000; Nagadhara *et al*., 2003); marker gene-free transgenic tobacco (Bharathi *et al*., 2011); chickpea (Chakraborti *et al*., 2009); rice (Yarasi *et al*., 2008) show effectiveness of *lectins* against sucking insect population. The efficacy of snowdrop (*G. nivalis*) and garlic lectins have been studied against phloem feeding *Aphis craccivora*, cotton aphids *A. gossypii* (Sauvion *et al*., 1996), red spider mite of tea (Roy *et al*., 2008), cotton leafworm and sap-sucking planthoppers (Powell, 2001). *Lectin* genes, as compared to the *Bt* genes are produced in storage parts of the plant as bulbs and in very minute amounts in roots and leaves. In this experiment semi-quantitative RT-PCR and

quantitative real-time RT-PCR showed differential expression of garlic *lectins* in garlic bulb, leaf, and root tissues. No difference in *lectin* expression levels could be observed between Chinese Purple and local garlic variety (Hazro). Bulb exhibited the highest expression level of lectin transcripts. This study reveals that garlic *lectin* genes are expressed in three tissues, bulb, leaf and roots. Predominant amount of the *lectins* are found in the bulb, being an important storage organ of the plant. Minor quantities of the *lectins* are found in leaf and roots. As the plant matures, concentration of the lectin reduces as it is used for plant growth. What could be the reason? It is speculated that expression levels are dependent on the maturation stages of respective tissues. Bulbs are storage organs thus *lectin* expression level is maximum in younger bulbs. Minor quantities of *lectin* are expressed in garlic leaves and roots. *Lectin* expression wears off with tissue maturation (Smeets *et al*., 1997).

Diversity in *lectin* genetic sequences leads to increased diversity in protein structure that defines their carbohydrate specificity (Barre *et al*., 1996). Lectin proteins are usually form dimers, homo, or hetero dimers for functions. Multiple alignments of *lectin* sequences obtained from different species revealed no well-defined conservation except patches of conserved block in the middle domain. Nonetheless, closely related species showed considerable homology in the entire length of the sequence. *Allium* species including garlic show increased similarity in *lectin* sequences. Thus, onion and garlic *lectins* are very closely related. Homology between the lectin proteins are more than 67% in the root and leaf lectin of garlic. Similarly, scattered topology obtained from phylogenetic reconstruction un-equivocally supports the diversity in the sequences. One of the reasons underlying the low conservation at inter-species level is the great difference in the size of sequences. While *Allium* species have the size of 300 to 600 bp, the maximum coding sequence length of the Viscum species is 1694. bp. The sequence size variations pose problems in alignments as well. The size variations may be due to the extensive exonization of introns events during the course of gene origination. Phylogenetic studies on the *lectin* gene sequences can further help in inferring the relationship of *lectin* sequences that lead to different lineages. Polymorphism and sequence diversity in lectin protein can also help in elucidating the amino acids and consequent protein domains that are recruited in increased insecticidal activities. If leucine is replaced by valine or aliphatic leucine, carbohydrate binding in lectins is abolished. The mannose binding sites are based on conserved sequence QXDXNXVXY.

Conclusion

In nutshell, the size variations in the coding sequence in the form of exonization of introns have played a significant role not only in sequence diversity in different species but also influenced their evolutionary relationship. The elevated expression of *lectin* genes in garlic bulb as compared to leaf and root tissues is evident from quantitative RT-PCR. The information generated from this study has implications in transforming insecticidal genes in crop plants against sucking insects.

References

Amjad, M., M.H. Bashir and M. Afzal, 2009. Comparative resistance of some cotton cultivars against sucking insect pests. *Pak. J. Life Soc. Sci.*, 7: 144–147

Aslam, M. and A.A. Gilani, 2000. Resistance of different cotton varieties to cotton leaf curl virus under field conditions. *J. Res. Sci*., 11: 42–45

Barre, A., E.J.M. Van Damme, W.J. Peumans and P. Rouge, 1996. Structure-function relationship of monocot mannose-binding lectins. *Plant Physiol*., 112: 1531–1540

Bharathi, Y., V.S. Kumar, I.C. Pasalu, S.M. Balachandran, V.D. Reddy and K.V. Rao, 2011. Pyramided rice lines harbouring *Allium sativum* (asal) and *Galanthus nivalis* (gna) lectin genes impart enhanced resistance against major sap -sucking pests. *J. Biotechnol*., 152: 63–71

Chakraborti, D., A. Sarkar, A.H. Mondal and S. Das, 2009. Tissue specific expression of potent insecticidal, *Allium sativum* leaf agglutinin (ASAL) in important pulse crop, chickpea (*Cicer arietinum* L.) to resist the phloem feeding *Aphis craccivora*. *Transgenic Res.*, 18: 529–544

Corbin, D.R., R.J. Grebenok, T.E. Ohnmeiss, J.T. Greenplate and J.P. Purcell, 2001. Expression and chloroplast targeting of cholesterol oxidase in transgenic tobacco plants. *Plant Physiol*., 126: 1116–1128

Damme, M.V.E.J., K. Smeets, S. Torrekens, V.F. Leuven, I.J. Goldstein and J.W. Peumans, 1992. The closely related homomeric and heterodimeric mannose-binding lectins from garlic are encoded by one-domain and two-domain lectin genes, respectively. *Eur. J. Biochem*., 206: 413–420

Ehsan, R. and Z. Altaf, 2009. Development, adoption and performance of Bt. cotton in Pakistan: A Review. *Pak. J. Agric. Res*., 22: 73–85

Foissac, X., N. Loc, P. Christou, A.M. Gatehouse and J.A. Gatehouse, 2000. Resistance to green leafhopper (*Nephotettix virescens*) and brown planthopper (*Nilaparvata lugens*) in transgenic rice expressing snowdrop lectin (*Galanthus nivalis* agglutinin; GNA). *J. Insect. Physiol*., 46: 573–583

Haq, S.K., S.M. Atif and R.H. Khan, 2004. Protein proteinase inhibitor genes in combat against insects, pests, and pathogens: natural and engineered phytoprotection. *Arch. Biochem. Biophys.*, 431: 145–159

Hussain, S.S., R. Makhdoom, T. Husnain, Z. Saleem and S. Riazuddin, 2008. Toxicity of snowdrop lectin protein towards cotton aphids *Aphis gossypii* (Homoptera, Aphididae). *J. Cell Mol. Biol*., 7: 29–40

Khan, M.R., I.U. Khan and G.M. Ali, 2012. MPF2-Like MADS-Box Genes Affecting Expression of SOC1 and MAF1 are Recruited to Control Flowering Time. *Mol. Biotechnol*., 54: 25–36

Kumar, S., N., Masatoshi, J. Dudley and K. Tamura, 2008. MEGA: A biologist-centric software for evolutionary analysis of DNA and protein sequences. *Brief Bioinform*., 9: 299–306

Liu, D., S. Burton, T. Glancy, Z.S. Li, R. Hampton, T. Meade and D.J. Merlo, 2003. Insect resistance conferred by 283kDa *Photorhabdus luminescens* protein TcdA in *Arabidopsis thaliana*. *Nat. Biotechnol*., 21: 1222–1228

Michiels, K., Van J.M. Damme and G. Smagghe, 2010. Plant-insect interactions: what can we learn from plant lectins? *Arch. Insect Biochem. Physiol*., 73: 193–212

Nagadhara, D., S. Ramesh, I.C. Pasalu, Y.K. Rao, N.V. Krishnaiah, N.P. Sarma, D.P. Bown, J.A. Gatehouse, V.D. Reddy and K.V. Rao, 2003. Transgenic indica rice resistant to sap-sucking insects. *Plant Biotechnol. J*., 3: 231–240

Pilobello, T., K. Krishnamoorthy, K.D. Slawek and K.L. Mahal, 2005. Lectin microarray for the rapid analysis of protein glycopatterns. *Chem. Biochem*., 6: 1–4

Powell, K.S., A.M.R. Gatehouse V.A. Hilder and J.A. Gatehouse, 1995. Antifeedant effects of plant-lectin an enzyme on the adult stage of the rice brown planthopper, *Nilaparvata lugens*. *Entomol. Exp. Appl.*, 75: 51–59

Powell, K.S., 2001. Antimetabolic effects of plant lectins towards nymphal stages of the planthoppers *Tarophagous proserpina* and *Nilaparvata lugens*. *Entomol. Exp. Appl.*, 99: 71–78

Ramachandraiah, G., R.N.A. Surolia and M. Vijayan, 2003. Computational analysis of multivalency in lectins: structures of garlic lectin–oligosaccharide complexes and their aggregates. *Glycobiology*, 13: 765–775

Ramzan, M., M. Afzal, A.S. Anwar and M. Hamid, 2008. Comparative efficacy of insecticides against sucking insect pests of cotton. *Pak. J. Life Soc. Sci*., 6: 140–142

Roy, A., D. Chakrabortia and S. Dasa, 2008. Effectiveness of garlic lectin on red spider mite of tea. *J. Plant Int*., 3: 157–162

Sadeghi, A., G. Smagghe, S. Broeders, J.P. Hernalsteens, H. De Greve, W.J. Peumans and E.J. Van Damme, 2008. Ectopically expressed leaf and bulb lectins from garlic (*Allium sativum* L.) protect transgenic tobacco plants against cotton leafworm (*Spodoptera littoralis*). *Transgenic Res*., 17: 9–18

Salman, M., A. Masood, Arif M.J.S. Saeed and M. Hamed, 2011. The resistance levels of different cotton varieties against sucking insect pests complex in Pakistan. *Pak. J. Agric. Agric. Eng., Vet. Sci*., 27: 168–175

Sauvion, N., H. Charles, G. Febvay and Y. Rahbé, 2004. Effects of jackbean lectin (ConA) on the feeding behaviour and kinetics of intoxication of the pea aphid, *Acyrthosiphon pisum*. *Entomol. Exp. Appl*., 11: 31–44

Sauvion, N., Y. Rahbé, J.W. Peumans, E.J.M. Van Damme, J.A. Gatehouse and M.A.R. Gatehouse, 1996. Effects of GNA and other mannose binding lectins on development and fecundity of the peach-potato aphid *Myzus persicae*. *Entomol. Exp. Appl*., 79: 285–293

Sengupta, S., D. Chakraborti, H.A. Mondal and S. Das, 2010. Selectable antibiotic resistance marker gene-free transgenic rice harboring the garlic leaf lectin gene exhibits resistance to sap-sucking plant hoppers. *Plant Cell Rep*., 29: 261–271

Smeets, K. J.M.W. Damme and J.W. Peumans, 1997. Developmental regulation of lectin and alliinase synthesis in garlic bulbs and leaves. *Plant Physiol*., 113: 765–771

Tajne, S., R. Sanam, R. Gundlab, S.N. Gandhic, L.R. Mancerac, R. Boddupally, R.D. Vudem and R.V. Khareedu, 2012. Molecular modeling of Bt Cry1Ac (DI–DII)–ASAL (*Allium sativum* lectin)–fusion protein and its interaction with aminopeptidase N (APN) receptor of Manduca sexta. *J. Mol. Graph Model*, 33: 61–76

Yarasi, B., V. Sadumpati, P.C. Immanni, R.D. Vudem and R.V. Khareedu, 2008. Transgenic rice expressing *Allium sativum* leaf agglutinin (ASAL) exhibits high-level resistance against major sap-sucking pests. *BMC Plant Biol*., 8: 102

(Received 10 June 2013; Accepted 12 July 2014)