CHAPTER 2.

REVIEW OF LITERATURE

2.1 Plant lectins

The term “hemagglutinins” or “phytoagglutinins” came into existence with the discovery of proteins possessing the ability to agglutinate erythrocytes. The first hemagglutinin capable of showing this property was reported in the castor bean extract (Stillmark, 1888). In 1940s, it was found that some of the haemagglutinins selectively agglutinated blood cells of a particular human blood group, therefore the novel term “lectin” was proposed by Boyd and Sharpleigh (1954) for the class of carbohydrate-binding proteins, which selectively agglutinate erythrocytes of particular human blood group. Lectins are carbohydrate-binding proteins that specifically recognize diverse sugar structures and mediate a variety of biological processes (Lis H, Sharon N 1998, Vijayan M, Chandra N 1999). Lectins are ubiquitous in nature, found in all kinds of organisms, from virus to humans (Sharon N 2008). Plant lectins are usually considered as a very heterogeneous group of proteins because comparative biochemical studies clearly indicate that they differ from each other with respect to their biochemical/physicochemical properties, molecular structure, carbohydrate-binding specificity and biological activities (Van Damme1998). Lectins can be produced by recombinant techniques. The natural sources and yields of some lectins are summarized in Table 1. The lectin contents in some parts of plants are higher, e.g., 390 and 75 mg of the purified lectin was recovered from 100 g Remusatia vivipara tubers (Bhat et al. 2010) and Astragalus mongholicus roots (Yan et al. 2005), respectively. Lectins are also found in seeds. The lectin content in nonlegume plants is low, e.g., 3.3 mg lectin from 100 g Hibiscus mutabilis seeds (Lam and Ng 2009). Lectins are found in abundance in legume seeds. Lectins are proteins, other than antibodies and enzymes, that bind specifically and reversibly to carbohydrates, resulting in cell agglutination or precipitation of polysaccharides and glycoconjugates.
### Table 2.1: Yields of plant lectins produced by recombinant DNA techniques

<table>
<thead>
<tr>
<th>Natural source of lectin</th>
<th>Lectin yield (mg/L culture medium)</th>
<th>Genetically modification in cells</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Allium sativum</em> (garlic) Leaf</td>
<td>5</td>
<td>cDNA was cloned into <em>Nde</em>I and <em>BamHI</em> restricted plasmid pET19b and expressed in <em>E. coli</em> strain BL21 (DE3) cells</td>
<td>Upadhyay et al. 2010</td>
</tr>
<tr>
<td><em>Artocarpus incise</em> (breadfruit)</td>
<td>16</td>
<td>cDNA was cloned into the pET-25b(+) and expressed in <em>E. coli</em>.</td>
<td>Oliveira et al. 2009</td>
</tr>
<tr>
<td><em>Artocarpus incise</em> (breadfruit)</td>
<td>18–20</td>
<td>cDNA was cloned into <em>EcoRI/XbaI</em> restricted plasmid pUC57 and expressed in <em>E. Coli</em></td>
<td>Oliveira et al. 2008</td>
</tr>
<tr>
<td><em>Glycine max</em> (Soybean)</td>
<td>0.1</td>
<td>cDNA was cloned <em>NcoI/NdeI/BamHI</em> restricted plasmid PET-3d and expressed in <em>E. coli</em> strain BL21(DE3)pLysS</td>
<td>Adar et al. 1997</td>
</tr>
<tr>
<td><em>Nicotiana tabacum</em> (tobacco) leaves</td>
<td>6</td>
<td>cDNA was cloned <em>EcoRI/NotI</em> restricted plasmid and expressed in <em>E. coli</em> strain top10F</td>
<td>Lannoo et al. 2007</td>
</tr>
<tr>
<td><em>Oryza sativa</em> (rice) roots</td>
<td>14.6</td>
<td>cDNA was cloned into <em>NdeI/BamHI</em> restricted pET 3D plasmid and expressed in <em>E. coli</em> strain BL21 (DE3) cells</td>
<td>Branco et al. 2004</td>
</tr>
<tr>
<td><em>Pisum sativum</em> (pea)</td>
<td>2–5</td>
<td>cDNA was cloned into <em>HindIII/PstI/BamHI</em> restricted plasmid and expressed in <em>E. coli</em> strain W3110</td>
<td>Stubbs et al. 1986</td>
</tr>
<tr>
<td><em>Polyporus squamosus</em> fruiting bodies</td>
<td>4–7</td>
<td>cDNA was cloned into <em>NdeI/BamHI</em> restricted plasmid and expressed in <em>E. coli</em> strain Nova Blue (DE3)</td>
<td>Tateno et al. 2004</td>
</tr>
</tbody>
</table>

Molecular cloning of lectins and lectin related products have led to new insight necessitating an update of definition of lectin. Some plant enzymes are fusion proteins composed of a carbohydrate binding and a catalytic domain. Similarly, type 2 RIPs such as ricin and abrin are fusion products of a toxic A-chain and a carbohydrate binding protein possesses only one binding site and therefore it is not capable of precipitating...
glycol-conjugates or agglutinating cells (Van Damme et al., 1994). In addition, several legume species contain proteins that are clearly related to the lectins but are devoid of carbohydrate-binding activity e.g. Phaseolus vulgaris, arcelins and α-amylase inhibitors (Mirkov et al., 1994). Keeping these facts in mind, Peumans and Van Damme (1995) defined plant lectins as all plant proteins that possess at least one noncatalytic domain that binds reversibly to a specific mono-or oligosaccharide. This new definition includes a broad range of proteins that have quite different agglutination and/or glyco-conjugate precipitation properties. Lectins are defined as carbohydrate binding proteins, which are highly specific for their carbohydrate moieties. These glycoproteins are of non-immune origin which reversibly binds the carbohydrate moiety and do not induce any change in the bound carbohydrate (Peumans et al., 1995). Lectins are extensively distributed in nature and several hundred of these molecules have been isolated from plants, fungi, viruses, bacteria, invertebrates and vertebrates (Wang et al., 2000a; Triueros et al., 2005). Lectins demonstrate anti-insect activity. They increase the mortality or delay the development of insect (Table 2).

Lectins are ubiquitous in biosphere having been found in plants, fungi, viruses, bacteria, insects and animals. Hundreds of different lectins have been isolated from plants. About 1-10% of the total soluble protein of legume seeds is composed of lectins (Peumans and Van Damme, 1998a). Based on evolutionary and structural relatedness, Peumans and Van Damme (1998b) placed the lectins in seven families: legume lectins, Chitin binding lectins, Type-2-ribosome-inactivating proteins (RIPs), monocot mannose binding lectins, amaranthins, cucurbitaceae phloem lectins and jacalin related lectins.

2.2 Classification of plant lectins

Various classifications have been given for plant lectins. Based on the overall structure, four major types of lectins are distinguished namely merolectins, hololectins, chimerolecins and superlectins. At present most accepted classification is based on evolutionary and structural relatedness of protein sequences and its groups i.e. lectin into seven distinct families (Peumans & Van Damme, 1999) e.g. the legume lectins, monocot mannose-binding lectins, jacalins, chitin binding lectins, type2 RIPs, amaranthins, and cucurbitaceae phloem lectins. Among all these the legume lectins are extensively studied and are present in several hundreds of species of legumes.
The overwhelming majority of plant lectins that have been isolated and characterized belong to different families and tribes of the *Leguminosae* family. Legume lectins are commonly glycosylated and composed of two or four protomers held together by non-covalent bonds, so that the functional lectin molecule has multiple carbohydrate binding sites. They also contain Mn$^{2+}$ and Ca$^{2+}$ ions associated with a series of highly conserved amino acids, which participate in carbohydrate binding. Various legume lectins may bind galactose, N-acetyl D-galactosamine, mannose, glucose, N-acetyl D-glucosamine, fucose, N-acetyl neuraminic acid, or more complex carbohydrates.

Chitin binding lectins contain one or more hevein domain; the term ‘hevein’ refers to a chitin binding polypeptide of latex of *Hevea brasiliensis*. They bind N-acetyl D-glucosamine oligomers and polymers of N-acetyl D-glucosamine.

Type-2-ribosome-inactivating proteins (RIPs) are lectins that catalytically inactivate ribosomes or eukaryotes and therefore irreversibly shut down protein synthesis. They bind galactose, N-acetyl galactosamine and N-acetyl neuraminic acid.

Monocot mannose-binding lectins bind only mannose and oligosaccharides of mannose and are found only in subgroups of monocot plants, alliaceae, amaryllidaceae, araceae, bromeliaceae, liliaceae and orchidaceae.

Legume lectins are a large family of homologous proteins which are confined to species of the plant family Leguminoseae. At present legume lectins have been purified from over 70 species belonging to different tribes, most from mature seeds. Legume lectins strongly differ from each other with respect to their carbohydrate-binding specificity (Sharon, & Lis, 1990). The majority of legume lectins belong to the mannose/glucose binding lectin (*Pisum sativum* lectin) and galactose/N-acetyl-galactosamine specific lectins (chickpea lectin).

### 2.3 Site of synthesis of legume lectin

Legume lectins accumulate in vacuoles and protein bodies. They are synthesized on the rough endoplasmic reticulum (RER) as preproteins or pre-proproteins. The precursor molecules are further processed by proteolytic cleavage of the polypeptides (pro-proteins to proteins) or trimming of sugar residues from the oligosaccharide side-chains of glycoproteins at the Golgi system (Fig1). The mature lectin molecule is then
transported via the endomembrane system to the site of accumulation (Chrispeels, 1984).

**Figure 2.1:** Schematic representation of the processing of one-chain and two-chain legume lectins (Mandal, et al., 1994)

### 2.3 Structure of legume lectin

Lectins exhibit similar structure as described before with slight differences such as they are calcium dependent and lack disulfide bonds. Legume lectin secondary structure is characterized by two anti-parallel beta sheets and the presence of tightly-bound calcium and manganese ions. They are devoid of alpha helices. Most conserved residues, such as the highly conserved Asp and Asn are located within the beta strands. Legume lectins have either two or four dome-shaped subunits, with the carbohydrate binding site existing as a shallow depression associated with the concave face of the curved beta sheet. Only one carbohydrate recognition domain (CRD) exists per subunit. The subunit molecular weights range from 25 to 30 kDa. The CRD of legume lectins is constructed of residues from four sequentially separate regions known as loops. Loops display variability in both sequence and size and all four binding site loops contain gaps. Asp and Gly comprise loops one and two, in which gaps are present beyond each residue. Asn and a hydrophobic residue are present in loop three. Additional interactions, usually with backbone atoms, from loop four complete the framework of carbohydrate recognition. The gaps in loops three and four are interspersed within the binding site residues. Mono-saccharides can bind only if they recognize and confirm to
two adjacent equatorial hydroxyl atoms present in the binding site. Specificity arises by additional variation in the binding site (Arason, 1996; Sharma and Surolia, 1997).

2.4 Role of lectins in plants

In spite of being the most thoroughly studied lectins, the functions of the plant lectins remain enigmatic. Proposed functions for plant lectins include a storage or transport role for carbohydrates in seed, binding of nitrogen fixing bacteria to root hairs and inhibition of fungal growth or insect feeding. All cells are coated with sugars and many also express surface lectins (Sharon, 1988). Cell surface sugars and lectins on cell surfaces are believed to function as recognition determinants between cells, either homotypic or heterotypic. For instance, the interaction between lectins on bacterial surfaces and sugars on eukaryotic cells play a crucial role in the infection process.

Lectins play a major role in nitrogen fixation in leguminous and non-leguminous plants. It has been suggested that root lectin recognized by bacterial receptor molecules is an important determinant of host plant specificity in *Rhizobium* legume symbiosis (Bohlool and Schmidt, 1978).

In addition to these roles, evidences exist for their involvement in embryogenesis, development and reproduction. Two lectin genes *MsLEC1* and *MsLEC2* from alfalfa cloned in antisense orientation and expressed in alfalfa lead to severe embryogenic, developmental and reproductive abnormalities (Brill *et al.*, 2001).

2.5 Plant lectins as plant defense proteins

Plant lectins effect various biological parameters of insects include larval weight decrease, mortality, feeding inhibition, delay in total developmental duration, adult emergence and fecundity on the first and second generation (Powell *et al.*, 1993; Habibi *et al.*, 1993). Plant lectins can either directly or indirectly cause profound morphological and physiological modifications in the insect intestine. In insects lectins bind to the midgut epithelium causing disruption of the epithelial cells including elongation of the striated border microvilli, swelling of the epithelial cells into the lumen of the gut lead to complete closure of the lumen and impaired nutrient assimilation by cells, allowing absorption of potentially harmful substances from intestine into circulatory system, fat bodies, ovarioles and throughout the haemolymph (Habibi *et al.*, 2000; Fitches *et al.*, 2001b).
Beside their insecticidal properties, lectins have also been found to be effective against transmission of plant viruses. Certain plant viruses are transmitted by insects of the *Hemiptera* order (sap-sucking insects) including aphids, whiteflies, leafhoppers, planthoppers, and thrips. The mode of transmission can either by moving from the alimentary canal of the vector insect onto its hemocoel and through the salivary secretory system into the plant host during insect feeding or by associating with the cuticular lining of the insect mouthparts or foregut and directly releasing as digestive secretions onto the plant when insect begins to feed (Gray *et al*., 2003; Hogenhout *et al*., 2008) (Fig 2). Lectins are able to recognize and bind to the viral glycoproteins thereby decreasing the binding of virus with the receptor and subsequently avoid the transport of virus from gut to hemocoel of insect vector (Desoignies, 2008), finally suspending the virus transmission.

The presence of lectins at relatively high concentrations in legume seeds has been associated with a possible role in plant defense. With the exception of some enzymes e.g. some type of chitinases, glucanases and glycosidases, lectins are the only plant proteins that are capable of recognizing and binding glycoconjugates present on the surface of microorganisms or exposed along the intestinal tract of insect or mammalian herbivores (Peumans and Van Damme, 1995). Molecular, biochemical, cellular, physiological and evolutionary arguments indicate that lectins have a role in plant defense. Most lectins are stable over a wide pH range, are able to withstand heat and are resistant to animal and plant proteases. Thus, they strongly resemble other defense related proteins such as some pathogenesis related proteins, protease inhibitors, chitinases, glucanases, RIPs, α-amylase inhibitors, antifungal proteins and thionins. Various plant lectins have shown insecticidal effects when fed to insects from coleoptera, homoptera, and lepidoptera.

Several plant lectins are protein inhibitors (*in vitro*) of animal and plant viruses which have glycoproteins in their virion (Balzanini *et al*., 1992). Binding of plant lectins to bacterial cell wall peptidoglycans indicate that lectins strongly interact with muramic acid, N-acetyl muramic acid and muramyl dipeptide and play direct role in plants defense against bacteria. The definitive proof for antifungal activity of plant lectins was demonstrated by a purified lectin from stinging nettle (*Urtica dioica*) which inhibited the growth of *Botrytis cinerea*, *Trichoderma hamatum* and *Phycomyces blakeslecanus* (Broekaert *et al*., 1989). Similarly, Hevein, a lectin from latex of rubber tree (Van Parijs
et al., 1991) and a lectin from seeds of *Amaranthus caudatus* (Broekaert et al., 1992) have antifungal activities.

### Table 2.2: Anti-insect activity of lectins (Sze Kwan Lam & Tzi Bun Ng, 2011)

<table>
<thead>
<tr>
<th>Natural source of lectin</th>
<th>Insect affected</th>
<th>Anti-insect effect</th>
<th>Sugar specificity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Allium sativum</em> (garlic) bulbs</td>
<td><em>Acyrthosiphon Pismum</em></td>
<td>Increased mortality</td>
<td>Mannose</td>
<td>Fitches et al. 2008</td>
</tr>
<tr>
<td><em>Arisaema intermedium</em> and <em>Arisaema wallichianum</em> (Araceae)</td>
<td><em>Bactrocera Cucurbitae</em></td>
<td>(1) Prolonged period of development (2) Inhibited pupation and emergence</td>
<td>Not found</td>
<td>Kaur et al. 2009</td>
</tr>
<tr>
<td><em>Gracilaria cornea</em> (red alga)</td>
<td><em>Boophilus Microplus</em></td>
<td>Reduced (1) the body weight of female after oviposition period, (2) the egg mass weight, and (3) hatching period</td>
<td>Fetuin, porcine stomach mucin</td>
<td>Lima et al. 2005</td>
</tr>
<tr>
<td><em>Gracilaria ornate</em> (red alga)</td>
<td><em>Callosobruchus Maculates</em></td>
<td>Delayed development</td>
<td>Fetuin, porcine stomach mucin</td>
<td>Leite et al. 2005</td>
</tr>
<tr>
<td><em>Xerocomus chrysenteron</em> fruiting bodies</td>
<td><em>Myzus persicae</em></td>
<td>Increased mortality</td>
<td>Fetuin, porcine stomach mucin</td>
<td>Jaber et al. 2008</td>
</tr>
<tr>
<td><em>Xerocomus chrysenteron</em> fruiting bodies</td>
<td><em>Myzus persicae</em></td>
<td>(1) Increased mortality (2) Reduction of body weight, duration of development and fecundity</td>
<td>Fetuin, porcine stomach mucin</td>
<td>Jaber et al. 2007</td>
</tr>
</tbody>
</table>
Janzen et al., (1976) for the first time reported on the toxicity of plant lectins to insects who attributed the inability of cowpea bruchid beetle *Callosobruchus maculatus*, to attack the seeds of *Phaseolus vulgaris* due to the presence of lectin (PHA). When the purified lectin was incorporated into artificial diet of insects at different concentrations, there was no insect survival at 5% level and even at 0.1%, the lectin caused a significant reduction in the number of larvae developing into adult hood. Ironically, this first indication for a protective role of lectins against insects was based on a false-positive result as the effect was due to a contaminating α-amylase inhibitor (Huesing et al., 1991). However, this observation appears to be specific to *P. vulgaris* and is not the case with lectin preparations from other species. Murdock et al., (1990) screened 17 plant lectins for their insecticidal activity on *C. macualatus*, five caused significant delay in larval development even at 0.1% level. The chitin-binding lectins from rice (*Oryza sativa*) and stinging nettle also inhibited larval growth of cowpea...
weevil (Huesing et al., 1991). These lectins though have insecticidal activity, their potential use in evolving transgenic plants becomes limited due to their mammalian toxicity.

Feeding trials with purified lectins from snowdrop (Galanthus nivalis) and garlic (Allium sativum) indicated that they are moderately active against chewing insects, such as cowpea weevil and tobacco hornworm (Spodoptera littoralis) but are not toxic to mammalian (Pusztai et al., 1992). Snowdrop lectin is the first to be effective on a sap sucking insect, rice brown plant hopper (Powell et al., 1993). Simiarly, Rahbe and Febvay (1993) have demonstrated that the lectin from Canavalia ensiformis (Con A) was a potent toxin of the pea aphid Acrystosiphon pisum with no toxicity on mammals. GNA caused 79% mortality to first-instar nymphs of rice brown plant hopper (Nilaparvata lugens) and 89% mortality to rice green plant hopper (Nephottetix cincticeps) whereas most other lectins had little or no effect (Powell et al., 1995).

GNA also caused mortality of pea aphids (Acrystosiphon pisum) and sugarcane whitegrubs (Antitrogus sanguineus) when incorporated into artificial diet (Allsopp and McGhie, 1996). Snowdrop lectin inhibited development and decreased fecundity of the glasshouse potato aphid (Aulacorthus solani) when administered in vitro and via transgenic plants (Down et al., 1996). Feeding trials of different mannose binding lectins on development and fecundity of peach-potato aphid (Myzys persicae) showed that snowdrop lectin was the most toxic (Sauvion et al., 1996). GNA also shows to antinutritive effects on lepidopteran, the tomato moth (Lacanobia oleracea) (Fitches and Gatehouse, 1998).

Machuka et al. (1999) carried out feeding assays with 25 lectins from 15 plant families on the development of legume pod borer, Maruca vitrata. Listera ovata agglutinin (LOA) and Galanthus nivalis agglutinin were effective against larval survival, weight, feeding inhibition, population, adult emergence and/or fecundity of Maruca pod borer larvae. According to Omitogun et al., (1999) artificial seeds (5%) significantly affected survival (1%) of C. macualtus, M. vitrata and C. tomentosicollis larvae. The purified African yam bean seed lectin also inhibited development of C. maculatus when larvae were fed on artificial cowpea seeds containing 0.2%, 2.0% and 5.0% of dietary lectin (Machuka et al., 2000). Macedo et al., (2002) have shown that Talisia esculent lectin also causes 90% mortality of C. maculatus and Zabrotes subfasciatus larvae.

Review and Literature…
2.6 Mechanism of action of lectins

The presence of lectins at relatively high concentrations in legume seeds has been associated with a possible role in plant defense. It has been demonstrated that some lectins bind to the brush-border membrane of the insect’s intestinal epithelial cells or to the pleiotrophic membrane in chitin-binding lectins. Other possible toxic effects include binding of lectins to glycosylated digestive enzymes. However, precise mechanism of action of lectins in insects is still unknown. Most lectins are stable over a wide pH range, are able to withstand heat and are resistant to animal and plant proteases. Thus, they strongly resemble other defense related proteins such as some pathogenesis related proteins, protease inhibitors, chitinases, glucanases, RIPv, α-amylase inhibitors, antifungal proteins and thionins. The toxicity of plant lectins to insects was first reported by Janzen et al., (1976), who attributed the inability of cowpea bruchid bettle Callosobruchus maculatus, to attack the seeds of Phaseolus vulgaris. When the purified lectin was incorporated into artificial diet of insects at different concentrations, there was no insect survival at 5% level. Also, even at 0.1%, the lectin caused a significant reduction in the number of larvae developing into adult hood (Huesing et al., 1991).

Wheat germ agglutinin (WGA) showed inhibitory effect on development of major pests of maize, the European corn borer (Ostrinia nubilalis) and the southern corn root worm (Diabrotica undecimpunctata) (Czapla and Lang, 1990). Feeding trials with purified lectins from snowdrop (Galanthus nivalis) and garlic (Allium sativum) indicated that they are moderately active against chewing insects, such as cowpea weevil and tobacco hornworm (Spodoptera litoralis) but are not toxic to mammalian (Pusztai et al., 1992). Snowdrop lectin was the first lectin to be effective on a sap sucking insect and rice brown plant hopper (Powell et al., 1993). GNA caused 79% mortality to first-instar nymphs of rice brown plant hopper (Nilaparvata lugens) and 89% mortality to rice green plant hopper (Nephrotettix cincteps) whereas most other lectins had little or no effect (Powell et al., 1995).

GNA also caused mortality of pea aphids (Acrithosiphon pisum) and sugarcane whitegrubs (Anitroges sanguineus) when incorporated into artificial diet (Allsopp and McGhie, 1996). Various plant lectins have shown entomotoxic effects when fed to insects from Coleoptera, homoptera & Lepidoptera orders.

Review and Literature…
2.7 Lectin genes

Biochemical studies, and sequence analysis of lectin genes from legumes reveals the presence of legume lectin gene families. Lectins have probably evolved through gene duplication and the carbohydrate binding domains of lectins have been incorporated to give them a protective role. The structures of lectin genes have been determined in soybean (Vodkin et al., 1983), french bean (Hoffman et al., 1982), pea (Kaminski et al., 1987), canavalia (Yamauchi and Minamikawa, 1990) etc. Hoffman et al. (1982) synthesized french bean cDNA using size fractionated mRNA. A cDNA library was constructed in pBR 322 vector at PstI site. After screening with a pea lectin cDNA probe, 8 positive clones were obtained indicating a considerable sequence homology. The sequence of two overlapping cDNAs complementary to lectin mRNA were compared and found to have no stop codon between the coding sequence for the β and α subunits. Soyabean seed lectin is under the control of one structural locus Le1, which is devoid of introns and produces a 1.0 kb mRNA. It codes for a signal sequence of 32 amino acids and a mature protein of 253 amino acids. The presumed promoter for the lectin gene (TATAATA) resembles a eukaryotic promoter consensus sequence and is found 27 bases upstream from the 5' end of the lectin message. Although soyabean seed lectin is produced by a single gene, another divergent gene Le2, homologous to Le1 has been identified (Goldberg et al., 1983). ConA, the gene coding for concanavalin A also lacks intron as reported for other legume lectin genes. There is multiple transcription initiation sites with the majority located in nucleotides 62-64 upstream from the initiation codon (Yamauchi and Minamikawa, 1990).

Molecular cloning of lectin mRNA of Phaseolus vulgaris indicated that the 5’ region of the lectin gene contains four AUG codons, one of which may initiate synthesis of a 20 residue long signal sequence and a mature protein of 223 amino acids (Hoffman et al., 1982). A genomic library of Phaseolus vulgaris DNA was screened using a lectin cDNA clone that included the entire coding region, to obtain a lambda clone containing the corresponding nuclear lectin gene. Comparison of the cDNA and genomic nucleotide sequence showed that the gene has no intervening sequence. The 5’ untranslated region was found to be only 10-16 base pairs long by S1 nuclease mapping. The lectin transcriptional unit is flanked by sequences that are exceptionally high (72-75%) in A + T (Hoffman, 1982).
Van Damme et al., (1991) constructed a cDNA library from total Poly (A)$^+$ rich mRNA isolated from snowdrop ovaries. Recombinant lectin clones were screened by colony hybridization using $^{32}$P end labelled oligonucleotide probe derived from a particularly known amino acid sequence for the lectin. A comparison of the lectin amino-acid sequence with the deduced amino acid sequence of a lectin cDNA clone revealed that lectin mRNA also encoded a 23 amino acid signal sequence and a C-terminus extension of 29 amino acids, besides the mature lectin polypeptide.

Lectin cDNA clones from Amaryllidaceae and Alliaceae species were analyzed by Van Damme et al., (1991) and have subsequently been used for plant transformation (Gatehouse et al., 1996). A cDNA clone encoding lectin was isolated by immunological screening of an expression library prepared from poly (A)$^+$ RNA from the inner bark of Robinia pseudoacacia. The cDNA clone had an open reading frame of 858 bp that encoded a polypeptide with a predicted molecular weight of 31210 Daltons (Yoshida et al., 1994). It appeared that lectin was synthesized as a precursor that consisted of a putative signal peptide of 31 amino acids and a mature polypeptide of 255 amino acids. Zhu et al., (1996) isolated a cDNA clone from a leaf cDNA library of Griffonia simplicifolia using polyclonal rabbit antibody. When it expressed in bacterial expression system, the recombinant protein exhibited N-acetyl glucosamine binding and insecticidal activity against cowpea weevil, indicating that glycosylation and multimeric structure are not required for these properties.

Gramineae lectins like WGA and barley lectins are synthesized as pre-pro-proteins; their signal peptides are removed co-translationally. The mature protein of all gramineae lectins that have been studied, consist of four homologous domains of 43 amino acids (Chrispeels and Raikhel, 1991), indicating that the genes arose through the duplication of a single domain. A characteristic chitin-binding domain is present in all the chitin binding lectins like hevein (Broekaert et al., 1989), tomato lectin (Kilpatrick, 1980), thorn apple lectin (Broakaert et al., 1987) and potato lectin (Allen et al., 1978; Desai et al., 1981). The presence of this common domain suggests that these proteins have evolved by gene fusion of the chitin-binding domain with unrelated domains.

Bauchrowitz et al., (1992) cloned and characterized two lectin genes from Medicago truncatula and designated them Mtlec1 and Mtlec2. The two genes show a high degree of homology and apparently belong to a small multigene family. Mtlec1 appears to encode a functional lectin with 277 amino acids, whereas Mtlec2 is probably
non functional. Since a frameshift mutation (insertion of two nucleotides) leads to premature translation termination after only 98 amino acids. The deduced amino acids sequence of the polypeptide Mtlec 1 suggests that this lectin is a metalloprotein with Glc/Man specificity.

Esteban et al., (2002) isolated a lectin gene, CanVLEC, from seedlings of chickpea (Cicer arietinum L. cv. Castellana) using cDNA library. CanVLEC is 981 bp and represents a full-length clone that encodes a polypeptide of 256 amino acid residues. The expression of the CanVLEC gene was specific in seedlings, mostly in hooks and elongating epicotyls, and no expression was detected in cotyledons and adult plants. The level of chickpea vegetative lectin transcripts in epicotyls decreased through the epicotyl’s growth suggesting a relationship to development.

Datta et al., (2000) constructed a cDNA library from developing seeds of cowpea in λZAPII vectors using poly (A)+ RNA. On primary, secondary and tertiary screening of the library using pea lectin probe, four clones were identified containing full-length lectin cDNA insert. One of these clones was sequenced completely that showed 85-92% homology with other legume lectin genes.

Ooi et al., (2001) cloned several complete cDNAs encoding the Narcissus tazetta lectins (NTL). The sequence analyses of the cloned DNAs revealed that there are at least three unidentical positive clones for NTLs. The primary structures of the three NTL clones contain a mature polypeptide consisting of 105 amino acids and a C-terminal peptide extension beyond the C-terminal amino acids Thr-Gly. There are two fixed position cysteines within the protein domain (amino acids 29 and 52), which are probably involved in the disulfide-bond linkage within the molecules to confer the secondary structure of the mature lectin. One third of the deduced amino acid composition consisted of glycine, leucine, and asparagine. From the cDNA derived amino acid sequences the three NTL clones were not identical and are suggested to be isolectins present in N. tazetta var. chinensis. This study further confirms the previous isolation of mannose-specific isolectins from Chinese daffodil leaves.

Chai et al., (2003) cloned a full-length cDNA of mannose-binding lectin gene sequence from a traditional Chinese medicinal herb Crinum asiaticum var. sinicum through RACE-PCR cloning. The full-length cDNA of C. asiaticum agglutinin (caa) was 820 bp and contained a 528 bp open reading frame encoding a lectin precursor
(preprotein) of 175 amino acid residues with a 22 aa signal peptide. The coding region of the caa gene was high in G/C content. The first 20 bp of the 5’ UTR had a GC content of 50%, which was a typical feature of the leader sequence. By cutting away the signal peptide, the CAA pro-protein was 15.79 kDa with a pl of 9.27 and contained 3 mannose-binding sites (QDNY). Random coil and extended strand constituted interlaced domination of the main part of the secondary structure. B-lectin conserved domain existed within N24 to G130. Predicted three-dimensional structure of CAA proprotein was very similar to that of GNA. It is significant that besides homology to known monocot mannose-binding lectins from Amaryllidaceae, Orchidaceae, Alliaceae and Liliaceae, caa also showed high similarity to gastrodianin type antifungal proteins. No intron was detected within the region of genomic sequence corresponding to the caa full-length cDNA. Southern blot analysis indicated that the caa gene belonged to a low-copy gene family. Northern blot analysis demonstrated that caa mRNA was constitutively expressed in all the tested tissue types including root, bulb, leaf, rachise, flower and fruit tissues.

A new lectin gene has been cloned from leaves of Arisaema heterophyllum using 5’ and 3’ RACE. The full-length cDNA of AHA was 1169 bp and contained a 777 bp open reading frame encoding a 258 amino acid protein. Analysis showed that AHA had high homology with many other mannose-binding lectins. Secondary and three-dimensional analyses showed that AHA had many common characters of mannose-binding lectin super family. Southern blot analysis of the genomic DNA revealed that aha belonged to a multigene family. Northern blot analysis demonstrated that aha constitutively expressed in various plant tissues including inflorescence, leaf and tuber, and highly expressed in the tuber (Zhao et al., 2003). Galasoo et al., (2004) amplified the part of a lectin gene encoding nucleotide sequences from lentil genomic DNA through PCR using primer designed from pea lectin and analyzed phylogenic relationship among various species of lentil.

A novel agglutinin gene was cloned from Arisaema lobatum using SMART RACE-PCR technology. The full-length cDNA of Arisaema lobatum agglutinin (ala) was 1078 bp and contained a 774 bp open reading frame encoding a lectin precursor (proprotein) of 258 amino acid residues with a 23 aa signal peptide. ALA shared varying identities, ranging from 40% to 85%, with mannose-binding lectins from other
species of plant families, such as *Araceae, Alliaceae, Iridaceae, Liliaceae, Amaryllidaceae* and *Bromeliaceae* (Lin J et al., 2005).

Chen et al., (2005) cloned the full-length cDNA of *Dendrobium officinale* agglutinin2 (DOA2) was cloned by rapid amplification of cDNA ends (RACE) using RNA extracted from *Dendrobium officinale* young leaves and primers designed according to the conservative regions of orchidaceae lectins. The full-length cDNA of DOA2 was 777 bp and contained a 513 bp open reading frame (ORF) encoding a lectin precursor of 170 amino acids. Semi-Quantitative RT-PCR analysis revealed that DOA2 mRNA expression was detected in all tested tissues including root, stem and leaf, however, the expression was higher in stem, and lower in leaf. As the DOA2 mRNA was detected in all the tested plant tissues, the DOA2 was considered to be a constitutively expressed gene.

Chen et al., (2005) cloned the full-length cDNA of *Zingiber officinale* agglutinin (ZOA) was by rapid amplification of cDNA ends (RACE) The full-length cDNA of zoa was 746 bp and contained a 510 bp open reading frame (ORF) encoding a lectin precursor of 169 amino acids with a signal peptide. ZOA was a mannose-binding lectin with three typical mannose-binding sites (QDNY). Semi-quantitative RT-PCR analysis revealed that ZOA expressed in all the tested tissues of *Z. officinale* including leaf, root and rhizome, suggesting it to be a constitutively expressing form.

Lucia Lioi et al., (2006) in some cultivated legume species, lectin protein coding genes were PCR amplified using primers designedon the basis of conserved N- and C-terminal amino acid sequences of the common bean (one-chain) or pea (two-chains) lectins. Amplification products of the expected length were obtained in *Lathyrus sativus* L., *Vicia faba* L. var. faba, *Phaseolus coccineus* L., and *Vigna unguiculata* (L.) Walp. No amplification product or agglutinating activity against blood cells, and/or cross-reaction with specific antibodies were detected in *Lupinus albus* L. and *Cicer arietinum* L. Finally, the new isolated nucleotide sequences, together with other legume lectin sequences already present in the EMBL Database, were used for evolutionary analysis. This last indicated the existence of two main clusters; one grouping all the species belonging to the Phaseoleae tribe and the other one grouping *Lens culinaris* Medik., *Pisum sativum* L., *L. sativus*, and *V. faba*, members of the *Vicieae* tribe. Results were congruent with the taxonomic classification and suggested that the lectin genes divergence in legume followed species evolution.

Review and Literature…
The cDNA (1124 bp) of *Alocasia macrorrhiza* was cloned by RACE-PCR with the deduced amino acid length of 270 and molecular weight is 29.7 kD. The results of homologous analysis showed a high similarity between AML and other mannose binding lectins and similar proteins from *Araceae* family. Two typical B-lectin domains and three mannose binding motifs were found in the sequence of AML.

A cDNA library was constructed in TriplEx2 vector using poly (A)+RNA from immature seeds of *Cicer arietinum*. The lectin gene was isolated from seeds of chickpea through library screening and RACE-PCR. The full-length cDNA of chickpea seed lectin (CpSL) was 972 bp and contains an 807 bp open reading frame encoding a 268 amino acid protein. Analysis shows that CpSL gene has strong homology with other legume lectin genes. Phylogenetic analysis showed the existence of two main clusters and clearly indicated that CpSL belonged to mannose-specific family of lectins. RT-PCR revealed that CAA gene expressed constitutively in various plant tissues including flower leaf root and stem (Qureshi et. al., 2007).

Tian et al., (2008) purified, characterised and cloned a novel mannose binding lectin from rhizomes of *Ophiopogon japonicas* with antiviral and antifungal activities. The full length cDNA of OJL contained 704 bp with an open reading frame encoding a precursor protein of 170 amino acid residues. Molecular modelling studies demonstrated that OJL exhibit a very similar three dimensional structure of the mannose binding sites with nother monocot mannose-binding lectins.

Peng et al., (2009) isolated a novel mannose binding lectin from *Clematis Montana* a traditional Chinese medicine with antiviral and apoptosis inducing activities. Nair et al., (2010) isolated two plant lectins with different sugar specificities were from the seeds of *Artocarpus integrifolia* and *Canavalia gladiata* using an affinity chromatography method.

2.8 Genetic engineering of plants for insect resistance

Conventional plant breeding has been quite successful in providing insect resistant varieties of certain crops. But in most of the cases it has not been possible to introduce resistance by this means, due to lack of variation in crossable material.

Genetic engineering of plants offer means by which traits can be freely transferred between species, providing that the genes that determine these traits can be isolated and transformed to crop plants. An advantage of using genetic engineering is

Genetic engineering allows development of crop varieties that are inherently resistant or tolerant of insect pests and thus offer following comparative advantages e.g. season long and weather independent protection, action only against harmful insects and at their most sensitive stages, biodegradable and ecologically safe material.

Developments in the field of plant tissue culture have enabled regeneration of insect resistant whole plants from the cells or tissues. Once whole plants have been obtained, they can be sexually or clonally reproduced in such manner that at least one copy of the sequence provided by the expression cassette is present in the cells of progeny. Alternatively once single transformed plant has been obtained, conventional plant breeding methods can be used to transfer resistance gene is in agronomically superior varieties via crossing and backcrossing. Such intermediate methods will comprise the further steps of:

1. Sexually crossing the insect resistance plant with plants from the insect susceptible taxon
2. Recovering reproductive material from the progeny of the cross
3. Growing insect resistance plants from the reproductive material

To substantially preserve the agronomic characteristics of the susceptible taxon repeated backcrossing with the insect resistant progeny must be desirable or necessary. Two types of insect control agents have been developed and proven effective following introduction into plants. The main approach uses δ-endotoxin coding sequences from the soil bacterium Bacillus thuringiensis. Bacillus thuringiensis crystal proteins were the first to be used to generate transgenic insect resistant crops (reviewed by Krattiger 1997). In another approach, proteins from microorganisms as well as plants have also been used for direct screening for insecticidal activities (Schulera et al. 1998). The
common higher plant defense proteins tested to date include lectins, protease, and α-amylase inhibitors (Duck and Evola 1997).

Bt toxin form an extensive range of preferred natural insecticides. Spores of Bt contain a crystalline protoxin which on ingestion by insects is cleaved by digestive protease in the insect gut to generate the active Bt toxin molecule (Choma et al., 1990). The active toxin molecules on binding to specific glycoprotein receptor on insect gut cells form ion channels which destroy the difference in ion concentration across the membrane resulting in death and lysis of the cells lining the gut (Manthavan et al., 1989). Bt toxin proteins are encoded by genes (cry) carried on a plasmid within the bacterium. Different strains of Bt contain plasmids encoding toxins with different sequences and different specificities of action against insects. Since the cloning and sequencing of the first insecticidal gene (Vaeck, 1987), more than 100 crystal protein gene sequences have been published. Whereas the isolation of genes encoding bt toxin was an easy task, subsequent of transgenic plants that express these toxins proved much less straightforward. In fact considerable modification to the Bt toxin gene has proved necessary in order to obtain adequate expression to confer insect resistance in transgenic plants. The necessary modification has fallen into two classes: alteration of the protein sequences of the Bt toxin and alteration to the gene sequence.

Expression levels of active toxin molecules were one to two order of magnitude higher when plants were transformed with truncated version of toxin gene that only encoded N-terminal region of the protein containing the active toxine gene. Genes encoding Bt toxins have been reconstructed by a combination of mutagenesis and oligonucleotide synthesis to produce synthetic genes which had codon usage typical for plant genes to enhance expression. Expression levels of Bt toxin from these synthetic genes was increased by nearly two orders of magnitude, measuring upto 0.3% of total protein (Perlak et al., 1991). The development of Bt transgenic plants was among the first biotechnology products of commercial relevance. Three commercial transgenic crops have been introduced that contain Bt toxin encoding gene for insect control: cotton, maize and potato. The first result concerning the transfer of Bt genes have been transferred to a number of other crops such as rice, cotton, soyabean, potato etc. with lepidopterans as the main target pests.

While transgenic plants harbouring Bt genes have been able to provide protection against several insect pests belonging to different orders, as more and more...
number of transgenics are put into field trial, there are widespread reports of resistance build up in insects. Higher plant genes encoding lectins and protease inhibitors of digestive enzymes in insects are viable alternative option for engineering insect resistance. The first gene of plant origin that was transferred to another plant species to result in enhanced insect resistance encoded a Bowman-Birk type serine protease inhibitor from cowpea (CpTi) (Hilder et al., 1987). A simple construct was prepared in which a full length coding sequence derived from a cDNA clone was placed under the control of the constitutively expressed cauliflower mosaic virus CaMV 35S promoter. Transgenic tobacco plants were produced by a standard Agrobacterium tumefacience mediated transformation protocol using a binary vector system. Transformants were screened for CpTi expression, which showed that many of the resulting plants expressed CpTi at levels greater than 0.1% of the total soluble protein. This was clearly in contrast to the very low levels of expression observed for unmodified toxin genes of bacterial origin. Other serine protease inhibitor genes have also been tested as protective agents for crops. For example the tomato inhibitor II gene when expressed in tobacco was also shown to confer insect resistance (Johnson et al., 1989). The wound inducible potato protease inhibitors (PI & PII) have been expressed in a range of crops where they have been shown to confer insect resistance (Mc Manus et al., 1994, Duan et al., 1996). The α-amylose inhibitor gene of phaseolus vulgaris under seed specific promoter has been expressed in transgenic tobacco. Seeds from the transgenic plants expressed the α-amylose inhibitor and contained inhibitory activity against mealworm, Tenebrio molitor (Altabella and Chrispeels, 1990). An efficient Agrobacterium-mediated genetic transformation of Brassica juncea (L.) Czern was done using leaf piece explants (Dutta et. al., 2008).

2.9 Transgenic plants expressing insecticidal lectin genes

In general, conferring resistance to plants for a susceptible insect the selected lectin should not be native to the plant, i.e., the lectin must come from a species other than the plant being transformed. However, in species that produce toxic lectins but not in sufficient amount as to kill insects, it may be preferable to insert a gene of the native lectin under strong constitutive promoter to cause over production, thus achieving insecticidal level. Alternatively, where a plant produces native lectin but the lectin is not
produced or not distributed to tissues which are normally infested by insects, a tissue specific promoter can be used to provide localized expression or over production.

A gene encoding pea lectin (P-lec) has been expressed at high levels in transgenic tobacco plants using the CaMV 35S promoter by Agrobacterium tumefaciens mediated transformation (Edwards, 1988). Pea lectin expressing plants were then tested in bioassay for resistance to the bollworm, Heliothis virescens. The results showed that leaf damage as well as larval biomass was reduced in transgenic plants (Boulter et al., 1990). Transgenic tobacco plants containing both the cowpea trypsin inhibitor gene (CpTi) and the pea lectin gene were obtained by cross breeding plants derived from the two primary transformed lines. These plants expressing the two insecticidal genes were also screened for enhanced resistance to H. virescens and the insecticidal effects of these two genes were found to be additive, the leaf damage being the least in the double-gene expressing plants. Hilder et al., (1995) developed transgenic potato plants expressing snowdrop lectin that were effective against peach-potato aphid (Myzus persicae). Transgenic potato plants expressing GNA at 0.3-0.4% of total soluble protein were toxic to the potato aphid, Aulacorthum solani (Down et al., 1996).

The incorporation of GNA in potato plants resulted in added protection against aphid Myzus persicae (Gatehouse et al., 1996) and tomato moth, Lacanobia oleracea (Gatehouse et al., 1997). The presence of GNA in transgenic rice plants expressing the protein at levels as high as 2% of the total soluble protein retarded development of the rice brown plant hopper (N. lugenes) and deterred its feeding (Rao et al., 1998).

Transgenic wheat plants containing the gene encoding snowdrop lectin under the control of constitutive and phloem specific promoters were generated through the particle bombardment method. Bioassay results showed that transgenic wheat plants from lines expressing GNA decrease the fecundity but not the survival of grain aphids (Sitobion avenae) (Stroger et al., 1999). Bioassay of Lacanobia oleracea (tomato moth) on ConA expressing potato plants showed that the lectin retarded larval development and decreased the larval weight by more than 45% but had no significant effect on survival. It also decreased the consumption of plant tissue by larvae. Con A expressing potato decreased the fecundity of peach-potato aphid (M. persicae) by up to 45%. Concavalin A, therefore, has potential as a protective agent against insect pests in transgenic crops (Gatehouse et al., 1999).
Three insecticidal genes (Bt genes Cry 1Ac and Cry 2A, and snowdrop lectin) were transferred into commercially important indica rice by particle bombardment. Bioassays using the triple transgenic plants showed 100% eradication of rice leaf feeder (Cnaphalocrocis medinalis) and yellow stem borer (Scirpophagea incertulas), and 25% reduction in the survival of the brown plant hopper (Nilaparvata lugens). The greatest reduction in insect survival, and the greatest reduction in plant damage, occurred in plants expressing all three transgenes (Maqbool et al., 2001).

Snowdrop lectin gene (gna) driven by phloem specific rice-sucrose-synthase promoter along with herbicide resistance gene (bar) driven by CaMV 35S promoter was employed for genetic transformation in indica rice. Plant progenies expressing gna exhibited substantial resistance against insect pests, brown planthopper (BPH) and green leafhopper (GLH) (Nagadhara et al., 2003).

The pea lectin gene under control of pollen specific promoter was introduced in oilseed rape (Brassica napus) for producing transgenic plants. Pea lectin expressed in anthers of transgenic T2-plants up to 1.5% of total soluble protein and it had negative correlation between lectin concentration and larval growth pattern of beetles (Melander et al., 2003). The development of peach-potato aphid was notably retarded when they were fed on transgenic tobacco plants expressing Helianthus tuberosus agglutinin (HTA) gene (Chang et al., 2003). Transgenic tobacco plants expressing Pinellia ternata agglutinin inhibited significantly the growth of peach-potato aphid (M. persicae). This study indicates that pta gene can be used as a supplement to the snowdrop lectin gene in the control of aphids (Yao et al., 2003).

Ramesh et al. (2004) transformed indica rice (Oryza sativa L.) with super-binary vectors containing Cry1Ab and Cry1Ac genes, and snowdrop lectin gene along with herbicide resistance gene. Transgenic lines expressing cry and gna exhibited substantial resistance against yellow stem borer as well as three major sap-sucking insects of rice. Nagadhara et al. (2004) obtained transgenic rice plants expressing snowdrop lectin (GNA) by Agrobacterium-mediated transformation. Transgenic lines expressing GNA exhibited high-level of resistance against the white backed plant hopper (Sogatella furcifera). Partial resistance to hemipterans has also been obtained by expression of a mannose-specific lectin from garlic (Allium sativum) leaves (ASA-L) in transgenenic rice (Saha et al., 2006) and a variety of other transgenic plant species.
Sadeghi et al., (2008) expressed leaf and bulb lectins from garlic (*Allium sativum* L.) protect transgenic tobacco plants against cotton leafworm (*Sporodoptera littoralis*). Recently, Chakraborti et al., (2009), reported phloem tissue specific expressions of *Allium sativum* leaf agglutinin (ASAL) gene, driven by rolC promoter in chickpea by *Agrobacterium* mediated transformation. The survival and fecundity of *Aphis craccivora* decreased to 11-26% and 22-42%, respectively when in planta bioassay conducted on T1 plants compared to untransformed control plant, which showed 85% survival.

Presently, the strategy employed by most of the workers is not only to use single genes, but also to use gene combinations whose products are targeted at different biochemical and physiological processes within the insect. These packages will not only contain lectin genes but also genes encoding other demonstrated insecticidal proteins such as enzyme inhibitors. The availability of suitable promoters and effective insecticidal genes means that the application of transgenic technology can now be extended to the control of almost any order of insects in near future. While several crops with commercial viability have been transformed in the developed world, however, progress has been minimal in the developing countries with harsh environments. There is a need to use these tools to provide resistance to insects in cereals, legumes, and oil seed crops for enhancing their productivity and meeting the demand for food for everyone. These are a source offered for increasing protection all around the world.