Plant Lectins and their Utilization for Development of Insect Resistant Transgenic Crop Plants

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Abstract

Lectins constitute a group of sugar-binding proteins which can recognize specific carbohydrate structures and are known to agglutinate various animal cells by binding to cell-surface glycoproteins and glycolipids. In diverse organisms, various biological processes are triggered and modulated by protein-carbohydrate recognition and protein-protein interactions. Lectins mediate cell-cell and host-pathogen interactions through the specific recognition of carbohydrates present on the cell surface. Moreover, lectins were found to exhibit resistance to heat denaturation and gut proteolysis, thereby affecting the entire bacterial population of the digestive tract. As such, lectins could exert profound effects on the metabolism and survival of various insects. The insecticidal activities of plant lectins against a wide array of insect pests belonging to homoptera, lepidoptera, coleoptera and diptera have been well documented. Transgenic crop plants expressing various lectins have been developed to confer protection against different pests.

Introduction

A toxic protein “ricin”, capable of agglutinating the RBCs of mammals, was identified, for the first time, from the castor beans (Stillmark, 1888). Initially, ‘agglutinins’ were identified only in plants followed by other organisms, and were termed as “lectins” because they agglutinated cells other than erythrocytes (Boyd & Slapeigh, 1954). Lectins are defined as proteins or glycoproteins of non-immune origin with one or more binding sites per subunit, which can reversibly bind to specific sugar segments through hydrogen bonds and Van Der Waals interactions (Lis and Sharon, 1998).

Based on primary structure, lectins are classified into four major groups:

1. Merolectins are monovalent, containing a single carbohydrate binding domain, which cannot precipitate glycoconjugates or agglutinate cells.
2. Hololectins are composed of at least two carbohydrate binding domains, identical or homologous, and bind structurally similar sugar(s). These proteins agglutinate cells and/or precipitate glycoconjugates owing to their multiple binding sites. Most of the plant lectins belong to this group.

3. Chimerolectins are fusion proteins comprising of one or more carbohydrate binding domain(s) arrayed in tandem with an unrelated domain. The unrelated domain shows well defined biological activity and functions independent of the carbohydrate binding domain. Chimerolectins can behave as merolectins or hololectins depending upon the number of carbohydrate binding domains.

4. Superlectins are also fusion proteins having two carbohydrate binding domains arranged in tandem, but differ in terms of structure and specificity.

Based on affinity to monosaccharide, plant lectins are also subdivided into five groups, viz., D-mannose/D-glucose; D-galactose/N-acetyl-D-galactosamine; N-acetyl-D-glucosamine; L-fucose; and N-acetylneuraminic acid-specific lectins.

**Classification of lectins based on sequence and three-dimensional structure**

Plant lectins are a heterogeneous group of carbohydrate binding proteins which differ in their molecular structure and carbohydrate binding specificity (Rini, 1995; Van Damme et al., 1998; Bouckaert et al., 1999; Barre et al., 2001). They occur in seeds and other tissues such as leaves, bark, stems, rhizomes, bulbs, tubers, etc. (Etzler, 1985, 1992; Peumans & Van Damme, 1998, 1999). Because of their broad distribution and ease of isolation, plant lectins constitute the best characterized group among different lectins. These proteins have been identified in about 500 plant species belonging to a limited number of plant families. Lectins that differ in their sugar-specificity have been found rarely in the same plant (Lis & Sharon, 1986). Plant lectins are considered as complex and comprise a heterogeneous group of proteins with varied biochemical/ physicochemical properties. Based on sequence and structural information, plant lectins are subdivided into seven families.

**Legume lectins**

These proteins are structurally and evolutionarily related to a well-defined group of lectins that were originally discovered in the seeds of legumes like jack bean, common bean, pea, peanut and soybean. The isolation and cloning of closely related agglutinins from the leaves of field balm (Glechoma hederacea) demonstrated that related lectins
also occur in the family laminaceae (Wang et al., 2003). Legume lectins exhibited fairly homogeneous molecular structure with ~30kDa subunits (Van Damme et al., 1998, 2007). Many of these lectins have been purified and characterized with respect to their structure, sugar-binding specificity and biological activity (Sharon & Lis, 1990; Van Damme et al., 1998; Peumans & Van Damme, 1999). These lectins shared extensive sequence homology and three dimensional structural similarities, but differed in carbohydrate specificity (Sharon & Lis, 1990; Rouge et al., 1991). Crystal structures of more than 15 legume lectins have been reported (Loris et al., 1998; Bouckaert et al., 1999), and the first legume lectin structure solved was that of concanavalin A (con A) (Hardman & Ainsworth, 1972; Baker et al., 1975). The structure belonged to ‘jelly-roll motif’ having a nearly flat rear six-stranded and a curved front seven-stranded β-sheets. Two metal ions, calcium and a transition metal, found in all the legume lectin structures are essential for the carbohydrate binding. The legume lectin fold was also found in some animal lectins such as galectins, pentraxins and a few other proteins from animal and microbial sources (Srinivasan et al., 1996).

ConA, pea, lentil and lathyrus lectins are dimers formed by side-by-side alignment of two monomers such that the two rear β-sheets form a contiguous 12-stranded β-sheet. However, this type of canonical dimer is missing in Ecorl, GS4 and peanut lectins. Also, dimers of these lectins possess back to back arrangement (‘hand shake dimer’) of the subunits (Srinivas et al., 2001).

All legume lectins were found to have similar carbohydrate-binding sites (Loris et al., 1998). The four loops A, B, C and D, associated with the concave face of the seven-stranded curved β-sheet at the top front-side of the subunit, form the binding site (Sharma & Surolia, 1997). The amino acid residues which bind Ca²⁺ and Mn²⁺ metal ions are highly conserved, while the residues which constitute the sugar-binding site are less conserved but exhibit similar properties (Lis & Sharon, 1998). The conserved Asp and Gly/Arg residues are present in loops A and B, whereas Asn and the hydrophobic residues (Phe/Tyr/ Trp/ Leu) are located in loop C. Size of the backbone of C loop determines carbohydrate specificity of the lectin. Aligned sequences of legume lectins showed 4 to 7 gaps in the binding loop D, indicating variation in the loop size (Sharma & Surolia, 1997) which contributes to broad specificity of legume lectins.

**Monocot mannose-binding (bulb) lectins**

Majority of the well-characterized plant lectins have been isolated from the seeds of dicotyledonous species. But lectins of non-seed origin from other species are also emerging as promising tools because of two reasons; 1) a good number of them might
contain novel sugar-binding sites; and 2) they can provide valuable information regarding the biological roles of plant lectins which still remain elusive. Also, non-seed lectins from monocotyledonous families, especially amaryllidaceae, have been identified (Cammue et al., 1986; Van Damme et al., 1987; 1991), which showed strict specificity for mannose (Van Damme et al., 1987; Shibuya et al., 1988; Van Damme et al., 1988) unlike other mannose/glucose-binding plant lectins. Mannose-binding lectins of monocots are present in bulbs and other vegetative tissues of plants belonging to amaryllidaceae, alliaceae, araceae, orchidaceae, liliaceae and bromeliaceae families (Van Damme et al., 1998).

A lectin with exclusive specificity towards mannose was isolated and characterized from snowdrop (Galanthus nivalis) bulbs (Van Damme et al., 1987) and was originally referred to as the ‘monocot mannose-binding lectin’ (Van Damme et al., 1998). Similar types of lectins have been identified in plants other than liliopsida, such as liverwort, (Marchantia polymorpha) as well as in bacteria and animals (Peumans et al., 2002). Hence, this group of lectins is now referred to as ‘GNA-related’ lectins after the first identified member, which share high similarity in sequence and overall structure (Van Damme et al., 1987; Barre et al., 1996). The monocot mannose-binding lectins exhibit the 'ß prism-II' fold (Wright, 1997). The crystal structure of snowdrop lectin (Galanthus nivalis agglutinin, GNA), complexed with α-D-mannose (Hester et al., 1995), showed the polypeptide chain organized into three sub-domains of flat, four-stranded anti-parallel β-sheet with a local three-fold symmetry, and is arranged to form three faces of a triangular prism. Strands of β-barrel run perpendicular to the barrel axis, while the axis itself coincides with the pseudo 3-fold axis. There are about 15 conserved hydrophobic side chains at the center of the barrel. Each of the β-sheets has a carbohydrate-recognition domain and share sequence homology (Van Damme & Peumans, 1991).

From garlic bulbs, two mannose-binding lectins, Allium sativum agglutinin ASAI (25kDa) and ASAII (48kDa), have been purified by affinity chromatography followed by gel filtration. The subunit structures of these lectins are different, but they showed similar sugar specificities (Fig 16.1). Both ASAI and ASAII are made up of 12.5 and 11.5kDa subunits. The constituent subunits of ASAI and ASAII exhibit the same sequence at their amino termini, and these proteins recognize monosaccharides in mannosyl configuration (Dam et al., 1998). The garlic lectin forms a heterodimer of two closely related subunits, and generates an additional site for binding 7 molecules of the substrate. In the case of daffodil lectin, there exists a fourth binding site which creates 16 substrate-binding sites per tetramer.
Cereal or chitin-binding lectins comprising hevein domains

The ‘hevein’ refers to a chitin-binding polypeptide of 43 amino acids found in the latex of rubber (*Hevea brasiliensis*) plants (Waljuno *et al.*, 1975). Many plant proteins owe their chitin-binding activity to the presence of one or more hevein domains. Moreover, it was shown that various lectins with hevein domains also have high affinity for N-glycosylated animal glycoproteins (Goldstein and Poretz, 1986). These lectins are widespread and occur both in monocot as well as dicot plant species.

The legume and cucurbitaceae phloem lectins also bind chitin but lack the hevein domain and have no sequence similarity with the chitin-binding lectins containing hevein domain. The chitin-binding lectins belong to the classes of merolectins, hololectins and chimerolectins. These lectins have been identified in plants belonging to graminaceae, solanceae, phytolaccaceae, urticaceae, papavaraceae and viscaceae (Raikhel *et al.*, 1993; Peumans *et al.*, 1996). Chitin-binding lectins are found in seeds as well as in other vegetative tissues. The lectins of graminaceae are the best characterized chitin-binding seed lectins, which showed marked specificity towards GlcNAc and GalNAc-oligomers. All graminaceae seed lectins are dimeric proteins composed of identical or similar protomers of around 17 kDa size. Wheat germ agglutinin (WGA), a representative member of chitin-binding lectins, exists in three isoforms (WGA1, WGA2, WGA3) that differ in 5-8 amino acid residues out of a total of 171 residues (Smith & Raikhel, 1989). High resolution
crystal structures for three isolectins in their native form have been solved (Wright, 1987, 1989; Harata et al., 1995). The 18 kDa WGA subunit is made up of four homologous domains of 43 residues each folded into a compact globule through four disulfide linkages and five or six β-turns, and the four domains are organized into a helical assembly. The monomers associate in a head-to-tail fashion to form dimers such that pairs of domains in contact are quasi two-fold related. In the contact regions of opposing monomers, four potential sugar-binding sites per dimer have been identified (Wright, 1990).

Jacalins
The family of jacalin-related lectins comprises all proteins with one or more domains that are structurally equivalent to jacalin, a galactoside-binding lectin present in jack fruit (Artocarpus integrifolia) seeds (Sastry et al., 1986). These proteins were sub-divided into two sub-families with a distinct specificity and molecular structure. The galactose-specific jacalin-related proteins comprise a small β (20 amino acid residues) subunit and a large α (133 amino acid residues) subunit, and exhibit a clear preference for galactose over mannose (Bourne et al., 2002). Whereas, subunits of mannose-specific jacalin-related lectins are built up of ~150 amino acids each, which exhibit exclusive specificity towards mannose. Lectins from moraceae (jacalin, MPA, artocarpin), convulvulaceae (calsepa, conarva), asteraceae (heltuba), gramineae (barley and wheat lectins) and musaceae (banana lectin) together constitute the Jacalin lectin family (Bourne et al., 1999). They often occur in seeds as well as in vegetative tissues, exhibit low sequence similarity and differ with respect to their carbohydrate binding specificity.

Type2-Ribosome inactivating protein (RIP) lectins with ricin-B domains
RIP lectins were earlier referred to as the family of ribosome-inactivating proteins that inactivate eukaryotic ribosomes through the removal of a conserved adenine residue from the ribosomal RNA (Van Damme et al., 2001; Stirpe, 2004). Based on their molecular structure, RIPs are subdivided into type-1, type-2 and type-3 classes. The B-chain of type-2 RIPs contains a lectin domain, referred to as the ricin-B-domain, named after ‘ricin’ (Lord et al., 1994).

These lectins are present in seeds and vegetative tissues of both monocot and dicotyledonous plants. They are chimerolecints composed of a polynucleotide adenosine glycosidase (PAG) domain called A-chain (Endo et al., 1987) linked to a galactose specific domain called B-chain (Lord et al., 1994). Type-2 RIPs share a high degree of sequence and structural similarity, but differ in catalytic activity, toxicity and carbohydrate-binding capacity. The ricin contains two subunits of 32 kDa held by disulfide (S-S) bridge and has specificity for Gal/GalNAc (Lord et al., 1994). Seeds of
rosary pea (*Abrus precatorius*) contain several isoforms of abrin, which is a heterodimer of 34 kDa and 32 kDa subunits joined together by single S-S bridge (Hedge *et al.*, 1991; Wu *et al.*, 2001). Similar type-2 RIPs have been isolated from the seeds of camphor tree, bitter gourd and mistletoe (Peumans & Van Damme, 1999). The two classes of lectins, type-2 RIPs and amaranthins, share the same ‘ß-trefoil fold’, which was first observed in the crystal structure of Kunitz soybean trypsin inhibitor (Murzin *et al.*, 1992).

**Amaranthins**

The amaranthin family is named after the *Amaranthus caudatus* seed lectin. These lectins are present in different species of amaranthus and all known amaranthins are homodimers built up of 33kDa subunits. Detailed specificity studies have shown that amaranthin preferentially recognizes the T-antigen disaccharide Galβ and GalNAc (Rinderle *et al.*, 1989), and is a homodimer having a ‘ß-trefoil fold’ involved in the carbohydrate binding (Transue *et al.*, 1997).

**Cucurbitaceae phloem lectins**

The family of cucurbitaceae phloem lectins is a small group of chitin-binding agglutinins found in the phloem exudates of a number of cucurbitaceae species (Sabnis and Hart, 1978). These lectins, also called as phloem proteins (PP2), are dimeric proteins built up of subunits of approximately 22kDa, and are specific for oligomers of GlcNAc (Wang *et al.*, 1994). Moreover, they showed high sequence similarity and are commonly found in some species of *Trichosanthes*, *Telfaira* and *Momordica*. Seeds of serpent cucumber (*Trichosanthes kirilowii*) contain a glycosylated galactose-specific lectin composed of disulfide-linked subunits of 37 kDa and 25 kDa (Falasca *et al.*, 1989). Similar seed lectins from snake gourd (*Trichosanthes anguina*) (Komath & Swamy, 1998) and common fringed-flower vine (*Trichosanthes cucumerina*) species (Padma *et al.*, 1999) have been isolated and characterized. Another seed lectin from fluted gourd (*Telfaira occidentalis*) is a hexameric protein with three pairs of disulphide-linked subunits of 30 kDa (Peumans & Van Damme, 1999). A galactose-specific seed lectin from bitter gourd (*Momordica charantia*) has also been characterized (Das *et al.*, 1981). Preliminary X-ray studies of lectins from serpent cucumber (Li *et al.*, 2000) and snake gourd (Manoj *et al.*, 2001) disclosed structural homology with type-2 RIPs.

**Inducible lectins**

Leaves of jasmonate-treated *Nicotiana tabacum* plants expressed a lectin nictaba, which provided evidence for the existence of inducible lectins (Chen *et al.*, 2002). Nictaba is a homodimer of 19 kDa subunits and exhibited specificity towards oligomers of N-
acetylglucosamine. Sequence analysis revealed that this lectin is a representative of a new family of inducible cytoplasmic proteins, and it disclosed similarity to the Cucurbitaceae phloem lectins. Immunolocalisation studies confirmed that nictaba is localized in the cytoplasm and the nucleus.

A mannose-binding lectin orysta was induced in rice plants by salt stress, desiccation, pathogen infection as well as jasmonic acid and abscisic acid. Orysta was shown to be a salt-inducible protein (Claes et al., 1990) and was later identified as a mannose-specific jacalin-related lectin. Since, it is synthesized only under specific stress conditions and is located in the cytoplasm and the nucleus, it is presumed to play a role in response to specific stress factors (Zhang et al., 2000).

**Lectin-carbohydrate specificity and interactions**

Although lectins were discovered >100 years ago, their involvement in carbohydrate recognition has been seriously dealt with only during the past two decades (Hughes, 1992; Lis & Sharon, 1998; Sharon & Lis, 1993). It is well known that all cells carry carbohydrates on their surface in the form of glycoconjugates (Cook et al., 1986), and lectins mediate cell-cell recognition by binding surface carbohydrates (Lis & Sharon, 1998). These cell-surface carbohydrates serve as points of attachment for other cells and pathogens. Sharon and Lis (1989) have proposed that the lectin-mediated cell-cell interaction takes place through different mechanisms, viz., 1) cell surface lectins bind to soluble glycoproteins and create inter-cell bridges; 2) lectins may bind carbohydrates of insoluble components of the extra-cellular matrix that promote cell adhesion; and 3) soluble lectins binding to carbohydrates on a pair of opposing cells may act as a bridge for these two cells.

The atomic basis of lectin-carbohydrate interactions has been elucidated through X-ray crystallographic analysis of various lectin-carbohydrate complexes. Forces which hold lectin-carbohydrate complexes together involved hydrogen bond networks, metal coordination, Van Der Walls and hydrophobic interactions (Drickamer, 1995; Elgavish & Shannan, 1997). Crystal structures of lectin-sugar complexes revealed various mechanisms for carbohydrate binding specificity, viz., 1) variation in the quaternary structure of legume and bulb lectins (Chandra et al., 1999; Vijayan & Chandra, 1999); 2) post-translational modifications of jacalin (Sankaranarayanan et al., 1996) and related lectins (Vijayan & Chandra, 1999); 3) water-molecule generated specificity in sugar complexes of PNA, Ecorl and other lectins (Ravishankar et al., 1997; Elgavish & Shannan, 1998); and 4) extended binding subsites and subunit multivalency (Elgavish & Shannan, 1997).
Plant lectins from edible sources

Lectins are present in the most commonly edible plant foods such as avocado, beans, beetroot, blackberries, cabbage, carrots, cherries, corn, garlic, leek, mushrooms, nuts, oregano, parsley, peanuts, peas, potato, rice, soybeans, spices, tea, tomato, wheat germ and also in diverse non-cultivated plant species (Nachbar and Oppenheim, 1980; Liener, 1986; Gupta and Sandhu, 1998; Oliveira et al., 2000; Leontowicz et al., 2001). Exposure of heterotrophic organisms, including humans, to functionally active lectins is highly common. The presence of nutritionally significant amounts of active lectins in fresh and processed foods, and lack of public knowledge concerning the deleterious effects of dietary lectins on the gut and overall health, have led to a number of outbreaks of food poisoning.

Many plant lectins have been found resistant to degradation by proteases in vitro (Carbonaro et al., 1997) and in the insect gut in vivo (Pusztai, 1991). Lectins, such as PHA (Pusztai et al., 1979; Hara et al., 1984), ConA (Nakata and Kimura, 1985), ConBr (Canavalia brasiliensis; brazilian jack bean) (Oliveira et al., 1994), PTA (Psophocarpus tetragonolobus; winged bean) (Higuchi et al., 1983), LEA (Lycopersicon esculentum; tomato) (Kilpatrick et al., 1985), GNA, SBA, WGA, PSA, SNA-I, SNA-II (Sambucus nigra; elderberry), VFL (Vicia faba; broad bean) and DGL (Dioclea grandiflora; mucuna) (Pusztai, 1991; Bardocz et al., 1995), were found resistant to in vivo breakdown by proteolytic enzymes. It is proposed that lectins are protected from proteolytic degradation during gut-passage perhaps as they bind to epithelial or luminal gut components. The protection observed from proteolytic degradation in vitro by adding glucose, Ca$^{2+}$ and Mn$^{2+}$ to the reaction mixture is consistent with this possibility. Nevertheless, because of the high resistance to proteolytic degradation in vivo, nutritionally significant amounts of certain dietary lectins will survive in an intact and highly reactive form within the gut lumen.

Most of the lectins are not degraded during their passage through the digestive tract as they bind to the exposed carbohydrate moieties present on the epithelial cells, which contribute to the toxicity of orally fed lectins. Indeed, lectins which are not bound by the mucosa, usually induce no harmful anti-nutritional effect (Pusztai and Bardocz, 1996). Once bound to the digestive tract, the lectin can cause dramatic changes in the cellular morphology and metabolism of the stomach and/ or small intestine and activate a cascade of signals, leading to altered intermediary metabolism.
Mechanism of toxicity and insecticidal properties of lectins

The insecticidal activity of plant lectins, against an array of insects belonging to homoptera, coleoptera, diptera and lepidoptera, have been well documented (Gatehouse et al., 1995; Schuler et al., 1998; Carlini and Grossi-de-Sa, 2002) (Table 16.1). Although the precise mode of action of plant lectins is not fully understood, it appears that resistance to proteolytic degradation by the insect digestive enzymes and binding to gut structures are two pre-requisites for lectins to exert their deleterious effects on insects. After binding to the surface of the intestinal epithelial cells, lectins interfere with the digestive, protective or secretory functions of the intestine. Most probably, binding of a lectin to the receptor decreases the absorption of nutrients and/or disrupt the midgut epithelial cells of insects (Gatehouse et al. 1984; Eisemann et al., 1994). Immunological studies, carried out to elucidate the mechanism of action of the mannose-specific lectin GNA on the rice brown planthopper (BPH), showed that no proteolytic degradation occurred either in the gut or honeydew of the insects fed on lectin-containing diet (Powell et al., 1998). Du et al. (2000) reported that ferritin acts as the most abundant binding protein for GNA in the midgut of BPH insects. Zhu-Salzmann et al. (1996) showed a correlation between receptor-binding and toxicity of griffonia (Griffonia simplicifolia) lectin towards the cowpea bruchid beetle. Similar results were reported against mustard aphid (Lipaphis erysimi) and cowpea aphid (Aphis craccivora) insects using the lectin isolated from the edible wild arum (Arum maculatum) tubers (Majumder et al., 2004). The resistance to proteolytic degradation and binding of ASAL to the gut receptors in the luminal epithelium of two major insect pests, mustard aphid (Lyphaphis erysimi) and red cotton bug (Dysdercus cingulatus), have been well documented (Bandyopadhyay et al., 2001).

A wide range of lectins, viz., GNA, Con A, PSA and ASA, exhibiting mannose or mannose/glucose sugar binding affinity, revealed palpable anti-metabolic effects towards members of the homopteran insects both under in vitro (Habibi et al., 1993; Powell et al., 1993; Rahbe et al., 1995) and in planta conditions (Powell et al., 1995; Gatehouse et al., 1996; Rao et al., 1998) (Table 16.2). Mannose-binding lectin GNA encoding gene has been introduced and expressed in diverse crop plants, viz., rice (Rao et al., 1998; Foissac et al., 2000; Tinjuangjun et al., 2000; Fitches et al., 2001a; Maqbool et al., 2001; Nagadharma et al., 2003, 2004), wheat (Stoger et al.,1999), tobacco (Hilder et al., 1995) and potato (Down et al., 1996; Sauvion et al., 1996; Gatehouse et al., 1996, 1997; Bell et al., 2001; Couty et al., 2001) to protect against different pests. Sun et al., (2002) reported enhanced resistance of transgenic rice carrying gna gene against the small brown planthopper. Similarly, A. sativum mannose-specific lectin (asa and asal) genes have been introduced into rice (Saha et al., 2006; Yarasi et al., 2008) and tobacco plants
(Bandyopadhyay et al., 2001; Sadeghi et al., 2007) which exhibited resistance against homopteran and lepidopteran pests.

Table 16.1 Toxicity of plant lectins against different groups of insects

<table>
<thead>
<tr>
<th>Lectin (plant source)</th>
<th>Insect</th>
<th>Host</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Mannose-specific lectins</td>
<td><em>Laodelpha striatellus</em> (rice small brown planthopper) <em>Nilaparvata lugens</em> (rice brown planthopper)</td>
<td>Rice</td>
<td>Powell et al., 1995</td>
</tr>
<tr>
<td>ASA, ASA I, ASA II and ASAL (<em>Allium sativum</em> agglutinin)</td>
<td><em>Myzus persicae</em> (peach potato aphid)</td>
<td>Peach, Potato</td>
<td>Sauvion et al., 1996</td>
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<td></td>
<td><em>Dysdercus cingulatus</em> (red cotton bug) <em>D. koenigii</em> (red cotton bug)</td>
<td>Cotton, Okra, Maize, Pearl millet</td>
<td>Roy et al., 2002</td>
</tr>
<tr>
<td></td>
<td><em>D. cingulatus</em>; <em>Lipaphis erysimi</em> (mustard aphid)</td>
<td>Cotton, Okra, Maize, Pearl millet</td>
<td>Bandyopadhyay et al., 2001</td>
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<td></td>
<td><em>Nilaparvata lugens</em> (BPH) <em>Nephotettix virescens</em> (GLH) <em>Sogatella furcifera</em> (WBPH)</td>
<td>Rice</td>
<td>Saha et al., 2006; Yarasi et al., 2008</td>
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<td></td>
<td><em>Myzus nicotianae</em> (tobacco aphid) <em>Spodoptera littoralis</em> Mustard aphid Peach potato aphid</td>
<td>Tobacco</td>
<td>Sadeghi et al., 2007 Sadeghi et al., 2008 Dutta et al., 2005a Dutta et al., 2005b</td>
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<td>CEA (Colocasia esculenta)</td>
<td><em>D. cingulatus</em>; <em>D. Koennigii</em></td>
<td>Cotton, Okra, Maize, Pearl millet</td>
<td>Roy et al., 2002</td>
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<tr>
<td>DEA (Differenbachia sequina)</td>
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<td>Cotton, Okra, Maize, Pearl millet</td>
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<td>GNA (Galanthus nivalis)</td>
<td><em>Callosobruchus maculatus</em> (bruchid weevil)</td>
<td>Cowpea</td>
<td>Gatehouse et al., 1991</td>
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<td><em>Acyrthosiphon pisum</em> (pea aphid)</td>
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<td><em>Antitrogus sanguineus</em> (sugarcane whitegrhie)</td>
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<td><em>Aulacorthum solani</em> (glasshouse potato aphid)</td>
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<td><em>M. persicae</em></td>
<td>Peach, Potato</td>
<td>Sauvion et al., 1996</td>
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<td><em>Lacanobia oleracea</em> (tomato moth)</td>
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<td>Fitches and Gatehouse, 1998; Fitches et al., 2001a</td>
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<td></td>
<td><em>Maruca vitrata</em> (legume pod-borer)</td>
<td>Cowpea</td>
<td>Machuka et al. 1999</td>
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Table 16.1 Contd...
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<table>
<thead>
<tr>
<th>Family</th>
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<th>Reference(s)</th>
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<td><strong>KPA</strong></td>
<td><em>Tarsophagus</em></td>
<td><em>Taro</em></td>
<td>Powell, 2001</td>
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<td><em>(Koelreuteria paniculata)</em></td>
<td><em>proserpina</em></td>
<td><em>Taro</em></td>
<td>Powell, 2001</td>
</tr>
<tr>
<td></td>
<td><em>L. striatellus</em></td>
<td><em>Rice</em></td>
<td>Loc et al., 2002</td>
</tr>
<tr>
<td></td>
<td><em>N. lugens</em></td>
<td><em>Rice</em></td>
<td>Powell et al., 1995, 1998; Loc et al., 2002; Nagadhar et al., 2003; Ramesh et al., 2004</td>
</tr>
<tr>
<td><strong>LOA</strong></td>
<td><em>Anagasta</em></td>
<td><em>Beans, grains, fruits, nuts</em></td>
<td>Macedo et al., 2003</td>
</tr>
<tr>
<td><em>(Listera ovata)</em></td>
<td><em>kuehniella</em></td>
<td><em>Rice</em></td>
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</tr>
<tr>
<td></td>
<td><em>M. vitrata</em></td>
<td><em>Cowpea</em></td>
<td>Machuka et al., 1999</td>
</tr>
<tr>
<td><strong>NPA</strong></td>
<td><em>N. lugens</em></td>
<td><em>Rice</em></td>
<td>Powell et al., 1995</td>
</tr>
<tr>
<td><em>(Narcissus pseudonarcissus)</em></td>
<td><em>M. persiaca</em></td>
<td><em>Peach, Potato</em></td>
<td>Sauvion et al., 1996</td>
</tr>
<tr>
<td><strong>Mannose/glucose specific lectins</strong></td>
<td><em>ConA</em></td>
<td><em>Pea</em></td>
<td>Rahbe and Febvay, 1993</td>
</tr>
<tr>
<td><em>(Canavalia ensiformis)</em></td>
<td><em>A. pisum</em></td>
<td><em>Pea</em></td>
<td>Rahbe et al., 1995</td>
</tr>
<tr>
<td></td>
<td><em>A. pisum</em></td>
<td><em>Pea</em></td>
<td>Rahbe et al., 1995</td>
</tr>
<tr>
<td></td>
<td><em>Aphis gossypii</em></td>
<td><em>Cotton, Mellon</em></td>
<td>Rahbe et al., 1995</td>
</tr>
<tr>
<td></td>
<td><em>(cotton and melon aphid)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Aulacorthum</em></td>
<td><em>Potato</em></td>
<td>Rahbe et al., 1995</td>
</tr>
<tr>
<td><em>(solani)</em></td>
<td><em>M. albifrons</em></td>
<td><em>(glasshouse and potato aphid)</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>(lupin aphid)</em></td>
<td><em>Lupin</em></td>
<td>Rahbe et al., 1995</td>
</tr>
<tr>
<td></td>
<td><em>Macrocephalum</em></td>
<td><em>Apple, Bean, Broccoli, Papaya</em></td>
<td>Rahbe et al., 1995</td>
</tr>
<tr>
<td><em>(albifrons)</em></td>
<td><em>M. persiaca</em></td>
<td><em>Peach, Potato</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>(potato aphid)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>L. oleracea</em></td>
<td><em>Tomato</em></td>
<td>Fitches and Gatehouse, 1998; Gatehouse et al., 1999; Fitches et al., 2001a</td>
</tr>
<tr>
<td></td>
<td><em>T. proserpina</em></td>
<td><em>Taro</em></td>
<td>Powell, 2001</td>
</tr>
<tr>
<td><strong>LCA</strong></td>
<td><em>A. pisum</em></td>
<td><em>Pea</em></td>
<td>Rahbe et al., 1995</td>
</tr>
<tr>
<td><em>(Lens culinaris)</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PSA</strong></td>
<td><em>A. pisum</em></td>
<td><em>Pea</em></td>
<td>Rahbe et al., 1995</td>
</tr>
<tr>
<td><em>(Pisum sativum)</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Hypera postica</em></td>
<td><em>(clover leaf weevil)</em></td>
<td>Elden, 2000</td>
</tr>
</tbody>
</table>

**Table 16.1**
<table>
<thead>
<tr>
<th>N-acetyl-D-glucosamine specific lectins</th>
<th>Plant</th>
<th>Insect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACA (Amaranthus caudatus)</td>
<td>Pea</td>
<td>A. pisum</td>
<td>Rahbe et al., 1995</td>
</tr>
<tr>
<td>BSA (Bandeiraea simplicifolia)</td>
<td>Corn</td>
<td>Diabrotica undecimpunctata (Southern corn rootworm)</td>
<td>Czapla and Lang, 1990</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ostrinia nubilaris (European corn borer)</td>
<td></td>
</tr>
<tr>
<td>BSAII</td>
<td>Pea</td>
<td>A. pisum</td>
<td>Rahbe et al., 1995</td>
</tr>
<tr>
<td>GSII (Griffonia simplicifolia)</td>
<td>Cowpea</td>
<td>C. maculatus</td>
<td>Zhu et al., 1996; Zhu-Salzman et al., 1998; Zhu-Salzman and Salzman, 2001</td>
</tr>
<tr>
<td>PAA (Phytolacca americana)</td>
<td>Corn</td>
<td>D. undecimpunctata; O. nubilaris</td>
<td>Czapla and Lang, 1990</td>
</tr>
<tr>
<td>TEL (Talissa esculenta)</td>
<td>Beans</td>
<td>C. maculatus; Zabrotes subfasciatus (Mexican dry bean weevil)</td>
<td>Macedo et al., 2002</td>
</tr>
<tr>
<td>WGA (Triticum aestivum)</td>
<td>Corn</td>
<td>D. undecimpunctata; O. nubilaris</td>
<td>Czapla and Lang, 1990</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Antitrogus sanguineus (sugarcane whitegrub)</td>
<td>Allsopp and McGhie, 1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H. postica</td>
<td>Eiden, 2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. erysimi</td>
<td>Kanrar et al., 2002</td>
</tr>
<tr>
<td>Galactose specific</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AHA (Artocarpus hirsuta)</td>
<td>Large number of grains</td>
<td>Tribolium castaneum (red flour beetle)</td>
<td>Gurjar et al., 2000</td>
</tr>
<tr>
<td>AIA (Artocarpus integrifolia)</td>
<td>Corn</td>
<td>D. undecimpunctata; O. nubilaris</td>
<td>Czapla and Lang, 1990</td>
</tr>
<tr>
<td>GHA (Glechoma hederacea leaf)</td>
<td>Potato</td>
<td>Leptinotarsa decemlineata (Colorado potato beetle)</td>
<td>Wang et al., 2003</td>
</tr>
<tr>
<td>RCA (Ricinus communis)</td>
<td>Corn</td>
<td>D. undecimpunctata; O. nubilaris</td>
<td>Czapla and Lang, 1990.</td>
</tr>
<tr>
<td>YBA (Sphenostylis stenocarpa)</td>
<td>Vigna spp</td>
<td>Clavigralla tomentosicollis (coreid bug)</td>
<td>Okeola and Machuka, 2001</td>
</tr>
<tr>
<td></td>
<td>Cowpea</td>
<td>C. maculatus; M. vitrata</td>
<td>Machuka et al., 2000</td>
</tr>
</tbody>
</table>

Table 16.1 Contd...
### N-acetyl-D-galactosamine specific lectins

<table>
<thead>
<tr>
<th>Lectin</th>
<th>Plant</th>
<th>Pest</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACA (Amaranthus caudatus)</td>
<td><em>A pisum</em></td>
<td>Pea</td>
<td>Rahbe <em>et al.</em>, 1995</td>
</tr>
<tr>
<td>BFA (Brassica fructiculosa)</td>
<td><em>Brevicoryne brassicae</em> (cabbage aphid)</td>
<td>Broccoli, Brussels sprouts, Cauliflower, Head cabbage</td>
<td>Cole, 1994</td>
</tr>
<tr>
<td>BPA (Bauhinia purpurea)</td>
<td><em>D. undecimpunctata</em>; <em>O. nubilaris</em></td>
<td>Corn</td>
<td>Czapla and Lang, 1990</td>
</tr>
<tr>
<td>CFA (Codium fragile)</td>
<td><em>D. undecimpunctata</em>; <em>O. nubilaris</em></td>
<td>Corn</td>
<td>Czapla and Lang, 1990</td>
</tr>
<tr>
<td>EHA (Eranthis hyemalis)</td>
<td><em>D. undecimpunctata</em></td>
<td>Corn</td>
<td>Kumar <em>et al.</em>, 1993</td>
</tr>
<tr>
<td>MPA (Maclura pomifera)</td>
<td><em>D. undecimpunctata</em>; <em>O. nubilaris</em></td>
<td>Corn</td>
<td>Czapla and Lang, 1990</td>
</tr>
<tr>
<td>PTA (Psophocarpus tetragonolobus)</td>
<td><em>C. maculatus</em>; <em>N. lugens</em></td>
<td>Cowpea</td>
<td>Gatehouse <em>et al.</em>, 1991</td>
</tr>
<tr>
<td>SNA-II (Sambucus nigra)</td>
<td><em>A pisum</em></td>
<td>Pea</td>
<td>Rahbe <em>et al.</em>, 1995</td>
</tr>
<tr>
<td>VVA Complex</td>
<td><em>D. undecimpunctata</em>; <em>O. nubilaris</em></td>
<td>Corn</td>
<td>Czapla and Lang, 1990</td>
</tr>
<tr>
<td>PHA (Phaseolus vulgaris)</td>
<td><em>L. hesperus</em> (Western tarnished plant bug)</td>
<td>Cotton, Alfalfa, Legumes</td>
<td>Habibi <em>et al.</em>, 2000</td>
</tr>
</tbody>
</table>

### GNA transgenics and their insecticidal activity

*G. nivalis* agglutinin (GNA), commonly known as snowdrop lectin, is a mannose-specific tetrameric protein, consisting of identical subunits of approximately 12 kDa (Van Damme *et al.*, 1987). GNA proved to be toxic to important insect pests belonging to the orders such as homoptera, coleoptera and lepidoptera (Gatehouse *et al.*, 1995, 1997). GNA protein was the most toxic among various lectins tested against BPH (Powell *et al.*, 1995). Immuno-histochemical localisation of GNA in intoxicated adults and nymphs disclosed that GNA could cross the insect-gut barrier (Powell *et al.*, 1998). The snowdrop lectin has exerted systemic effects via transport from the gut contents to the haemolymph across the gut epithelium (Fitches *et al.*, 2001a). Accordingly, the mechanism of action of
GNA toxicity seems complex with various target sites for its action, which plausibly contribute to delayed development of resistance by the insects. As such, deployment of gna gene has an added advantage for the development of durable pest resistance in crop plants.

In view of the marked entomotoxic effects exhibited by the GNA, it has been introduced into a number of dicot and monocot plants. The gna was initially expressed in tobacco and mustard, and the transgenic plants showed increased resistance against aphids (Hilder et al., 1995; Goswami et al., 1998). Transgenic potato plants expressing GNA disclosed resistance against potato aphid (Down et al., 1996), potato peach aphid (Gatehouse et al., 1996; Sauvion et al., 1996) and a lepidopteran tomato moth (Gatehouse et al., 1997; Bell et al., 2001). It was reported that transgenic rice containing gna conferred substantial resistance against BPH insects (Rao et al., 1998). Similarly, expression of GNA in wheat, conveyed resistance against the wheat grain aphid (Stoger et al., 1999). In peach potato aphids, the mannose-binding GNA and ConA lectins reduced the uptake and absorption of nutrients (Sauvion et al., 1996). Fitches and Gatehouse (1998) demonstrated that GNA and ConA bind to the soluble brush-border-membrane enzymes in the midgut of tomato moth (Lacanobia oleracea). Indica rice varieties transformed with gna by particle-bombardment method (Sudhakar et al., 1998; Foissac et al., 2000; Maqbool et al., 2001) and by Agrobacterium-mediated method (Nagadhara et al., 2003, 2004) showed enhanced levels of resistance against major sap-sucking insects. Apart from homopteran pests, GNA also caused toxic effects against larval growth and development of several lepidopteran insects including tomato moth (L. oleracea), Mexican rice borer (Eoreuma loftini), sugarcane borer (Diatraea saccharalis) and bollworm (H. armigera) (Gatehouse et al., 1997; Setamou et al., 2003; Shukla et al., 2005).

Transgenic rice plants, expressing GNA, showed significant resistance towards BPH, GLH and WBPH insects with minimal plant damage, and exhibited high levels (1-2 point score on 0-9 scale) of resistance to BPH, GLH and WBPH insects on a par with those of known resistant checks (Nagadhara et al., 2003, 2004). The expression of GNA in transgenic rice caused various insecticidal effects such as decreased feeding and declined insect survival similar to those of GNA fed to BPH and GLH in artificial diets (Powell et al., 1993). The survival of BPH/GLH/WBPH nymphs fed on homozygous GNA transgenic plants was reduced to 55%, 49% and >90%, respectively, as compared to the nymphs fed on control plants. This decline in the nymphal survival is attributable to the selective expression of GNA in the phloem sap of transgenic plants, since gna is driven by the phloem-specific RSs1 promoter. However, a few insects which survived on GNA transgenics showed delayed moulting and life cycle when compared to the insects fed on untransformed control plants. Marked decreases were observed in the honeydew production of BPH (80%), GLH (69%) and WBPH (90%) insects when fed on GNA-transgenic rice plants, owing to the high anti-feedant effect of GNA against sucking insects (Nagadhara et al., 2003, 2004).
Table 16.2 Plant lectin genes deployed for development of transgenic plants confer resistance against different insects

<table>
<thead>
<tr>
<th>Lectin</th>
<th>Transgenic crop</th>
<th>Target pest</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>WGA</td>
<td>Maize</td>
<td>Ostrinia nubilaris; Diabrotica undecimpunctata</td>
<td>Maddock et al., 1991</td>
</tr>
<tr>
<td>WGA</td>
<td>Mustard (B. juncea)</td>
<td>Lipaphis erysimi</td>
<td>Kanrar et al., 2002</td>
</tr>
<tr>
<td>PHA-E&amp; L</td>
<td>Arabidopsis thaliana</td>
<td>Lacanobia oleracea</td>
<td>Fitches et al., 2001b</td>
</tr>
<tr>
<td>GNA</td>
<td>Potato</td>
<td>Aulacorthum solani</td>
<td>Down et al., 1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Myzus persicae</td>
<td>Gatehouse et al., 1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Couty et al., 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. oleracea</td>
<td>Fitches et al., 1997</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>Gatehouse et al., 1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. oleracea</td>
<td>Bell et al., 1999, 2001; Down et al., 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aphidius ervi (parasitoid of M. persicae)</td>
<td>Couty et al., 2001</td>
</tr>
<tr>
<td>GNA</td>
<td>Rice</td>
<td>Nilaparvata lugens (Brown planthopper)</td>
<td>Rao et al., 1998; Foissac et al., 2000; Tinjuangjun et al., 2000; Maqbool et al., 2001; Tang et al., 2001; Loc et al., 2002; Nagadhar et al., 2003; Ramesh et al., 2004.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nephotettix virescens (Green leafhopper)</td>
<td>Foissac et al., 2000; Nagadhar et al., 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cnaphalocrocis medinalis (rice leaf folder); Scirpophaga incertulas (yellow stem borer)</td>
<td>Maqbool et al., 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sogatella furcifera (Whitebacked planthopper)</td>
<td>Nagadhar et al., 2004</td>
</tr>
</tbody>
</table>

Table 16.2 Contd...
Insecticidal activity of ASAL transgenics

It was shown that the characteristic odour of *Allium* species, including onion and garlic, is primarily due to the enzymatic degradation of sulfur-containing amino acids. The enzyme allinase involved in this process is known to represent an important fraction of the total protein content of *Allium* bulbs and leaves (Fowden, 1964). Since then numerous reports appeared on the occurrence, purification and characterization of allinases from several
alliaceae species (Tobkin and Mazelis, 1979; Nock and Mazelis, 1986, 1987; Fugita et al., 1990). Isolation and characterization of mannose-specific lectin from *A. sativum* (garlic) was carried out by Van Damme et al. (1991). In comparison with amaryllidaceae lectins which are composed of identical 12 kDa subunits, garlic lectins consist of two different subunits of 11.5 kDa and 12.5 kDa referred to as ASA1 (heterodimer) and 12 kDa subunits in ASAII (homodimer). Transgenic plants expressing ASA and ASAII showed reduction in the larval weight of *S. littoralis* (Fitches et al., 1997). Since ASA and ASAII adversely affected the larval weight gain, their development into pupae was retarded. According to Bandyopadhyay et al. (2001), ASAL binds to the carbohydrate part of the 55 and 45 kDa brush-border-membrane vesicle receptor proteins of mustard aphid (*Lyphaphis erysimi*) and red cotton bug (*Dysdercus cingulatus*), respectively; it was suggested that binding of ASAL to these receptors decreases the permeability of the membrane. Earlier studies disclosed that the garlic-bulb lectin ASA induces significant anti-metabolic effects towards third instar nymphs of the rice brown planthopper (BPH) when fed on the artificial diet (Powell et al., 1995). Roy et al. (2002) reported that ASA causes high mortality of ~78% against the red cotton bug (*Dysdercus cingulatus*) when added to the artificial diet. ASAL-transgenic tobacco plants exhibited insecticidal activity against the mustard aphid (Dutta et al., 2005a), and also reduced the survival and fecundity capacity of the peach potato aphid (Dutta et al., 2005b). Sadeghi et al. (2007) described the insecticidal activity of ASAL and ASAII proteins in transgenic tobacco plants against the tobacco aphid (*Myzus nicotianae*). Transgenic tobacco expressing ASAII and ASAL exerted significant detrimental effects on the larval development, growth and survival of the cotton leaf worm (Sadeghi et al., 2008). Transgenic rice expressing ASAL exhibited moderate to high-level resistance against homopteran BPH, GLH and WBPH insects (Saha et al., 2006; Yarasi et al., 2008).

Transgenic rice plants expressing ASAL, conveyed explicit resistance towards BPH, GLH and WBPH insects with minimal plant damage (Fig 16.2). After infestation, insects surviving on ASAL-transgenic plants varied from 2 to 4/plant which exhibited delayed moulting and prolonged life cycle (~10 days) compared to the insects fed on susceptible control plants. The survival of BPH was reduced by 74% to 83% on ASAL-transgenic rice lines as compared to the control plants. Similarly, GLH survival was decreased by 79% to 84% on transgenic lines when compared to the controls. Likewise, the survival of WBPH was reduced by 64% to 77% on transgenic lines in comparison with the susceptible control plants (Yarasi et al., 2008). The insect bioassay results suggest that the ASAL-expressing transgenic lines exhibit a distinctly higher-level of resistance against both BPH and GLH pests compared to that of GNA-transgenics. Whereas, WBPH nymphs fed on GNA-transgenic rice showed 90% mortality compared to 70% mortality observed on ASAL-transgenics (Yarasi et al., 2008). Furthermore, insect fecundity assays on ASAL rice lines revealed significant decreases in the nymphal production of BPH
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(66%), GLH (69%) and WBPH (65%) insects, resulting from marked decreases in the fecundity of BPH, GLH and WBPH insects. The accrued results amply indicate the high antifeedant and severe entomotoxic effects of ASAL on these pests. Marked decreases of 92 to 94%, 80 to 83% and 60 to 68% were observed in the honeydew production of BPH, GLH and WBPH insects, respectively, when insects were fed on ASAL-transgenic plants (Yarasi et al., 2008). An overview of insect bioassays demonstrated that ASAL is more toxic to BPH and GLH insects compared to GNA. Whereas, GNA exhibited higher toxicity to the WBPH than ASAL under similar bioassay conditions. The variable entomotoxic effects of GNA and ASAL proteins on three sap-sucking pests are attributed to their differential binding affinities to receptor proteins on the gut epithelial cells of insects.

![Fig 16.2 Evaluation of transgenic rice line expressing ASAL against Brown Planthopper (BPH)](image)

**Pyramiding of lectin transgenes**

Preventing insect pests from developing resistance to cultivars poses a major challenge for achieving sustainable crop productivity. Introgression of different combinations of resistance genes into the genetic milieu of a cultivar serves as an effective strategy for achieving durable and broad-based resistance against various insects/pathogens (Liu et al., 2000). Rice transformants stacked with gna, cry1Ac and cry2A genes disclosed higher levels of insect resistance compared to the transgenic plants expressing single genes (Maqbool et al., 2001). Survival of BPH/GLH/WBPH insects was reduced by 86%/85%/90% on pyramided lines compared to 77%/80%/60% on ASAL and 57%/56%/83% on GNA parental transgenics, respectively. Insect bioassays conducted on
pyramided lines revealed notable decreases in the nymphal production of BPH (76%), GLH (75%) and WBPH (90%) when compared to ASAL (72%, 70% and 72%) and GNA expressing (56%, 55% and 86%) parents, suggesting substantial decreases in the fecundity of insects fed on pyramided plants. Furthermore, marked decreases were observed in the honeydew production of BPH (93%), GLH (93%) and WBPH (95%) fed on pyramided rice lines compared to 93% (BPH), 91% (GLH) and 87% (WBPH) on ASAL lines, and 90% (BPH), 87% (GLH) and 91% (WBPH) on GNA plants, indicating the enhanced toxicity of pyramided lectins on the feeding capacity of insects. *In planta* insect bioassays, revealed that pyramided (asal+gna) rice lines are more effective in reducing insect survival, fecundity, feeding ability and development of three major sap-sucking pests. The increased resistance imparted by the pyramided lines against these insects is attributable to the differential binding affinities of ASAL and GNA proteins to the gut-epithelial cells of insects, resulting form synergic entomotoxic effects (Bharathi, 2009).

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