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Development of entomotoxic molecules as control agents: illustration of some protein potential uses and limits of lectins (Review)

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Worldwide use of pesticide has dramatically increased during the last two decades, which are known to cause a lot of problems to both environment and human health. Due to the harmful effects of insecticide on environment, human health, non target organism, water pollution and increasing insect resistance, recent efforts have been made to reduce broad spectrum of toxicant added to the environment. It is necessary to balance agricultural needs with environmental and health issues when using insecticides. Currently, the use of entomotoxic proteins has been increased because of the growing need to obtain better food quality and to protect the environment. Bt toxins derived from bacteria, *Bacillus thuringiensis* (Bt) the main commercial genetically modified organism (GMO), or entomotoxic product were successfully used against a range of insect orders such as Lepidoptera, Coleoptera and Diptera. In recent years, several investigations were focused on other entomotoxic potential, including products based on some defense proteins present in plants. Among them some classes of proteins such as digestive enzyme inhibitors and lectins showed greater potential for exploitation in transgenic-based pest control strategies. Currently, some lectins from plant and fungal origin were successfully used against a wide range of important insect pests.
Keywords. Protein, lectins, plant-insect interactions, ribosome-inactivating proteins, proteases inhibitors, amylase inhibitors.

Développement de molécules entomotoxiques comme agents de contrôle : utilisations potentielles de certaines protéines et limites des lectines (Revue). L'utilisation des pesticides à travers le monde a augmenté de façon spectaculaire ces vingt dernières années. Pourtant, les effets que ceux-ci peuvent avoir vis-à-vis de la santé humaine et de l'environnement ont déjà été largement mis en évidence par de nombreux auteurs. Pour réduire l'utilisation de substances toxiques, des efforts ont été concentrés sur le développement d'alternatives biologiques. Pendant plusieurs décennies, les toxines produites par la bactérie *Bacillus thuringiensis* (Bt) ont été le seul principe actif intégré dans des organismes génétiquement modifiés (OGM) de grandes cultures afin de conférer des résistances aux insectes ravageurs. Ces dernières années, plusieurs recherches ont été menées sur le développement de nouvelles molécules insecticides, notamment des protéines de défense produites naturellement dans certaines plantes. Parmi ces protéines se trouvent, entre autres, des inhibiteurs d'enzymes digestives et des lectines. Une des méthodes prometteuses pour accroître la résistance des plantes serait de valoriser en particulier le potentiel des lectines d'origine végétale ou fongique vis-à-vis des ravageurs.
Mots-clés. Protéine, lectines, interactions plante-insecte, plantes transgéniques, protéines inhibitrices de la fonction ribosomiale, inhibiteurs de protéases, inhibiteurs d'amylase.

1. INTRODUCTION

Due to deleterious effects of chemical pesticides on the environment and non target organisms, recent efforts have been made to reduce the systematic application of pesticides and to replace them with other methods. Hence, there has been a need to discover new effective pests control methods conferring a protection to plants against insect pests (Conner et al., 2003; Majumder et al., 2005; Ferry et al., 2006; Macedo et al., 2006). First effort for this purpose was to develop and increase the use of protein toxins from *Bacillus thuringiensis* (Bt). This bacterial protein was widely used as bio-insecticide against a wide range of insect pests because it has little effect on the other organisms and environment. Indeed, this method was first started commercially in 1950s with Bt toxins as biological insecticides (Aronson, 1994; Federici, 2005). Bt preparations were initially sprayed directly for several decades on plants to protect them against a variety of insect pests, but in 1981 the genes encoding the pesticide proteins have also been successfully transferred into plant species such as maize, providing protection without the need for spraying (Schnepf et al., 1981; Federici, 2005).

Currently, due to resistance of some insect pests to repeated Bt toxin applications (Tabashnik et al., 1990; McGaughey et al., 1992; Ferre et al., 2002; Janmaat et al., 2003), and none affected homopteran insect pests by Bt toxins, other supplemented methods, including the use of entomotoxic proteins were introduced (Bandyopadhyay et al., 2001; Carlini et al., 2002; Vasconcelos et al., 2004; Wang et al., 2006). To date, several different classes of plant proteins including lectins, ribosome-inactivating proteins, protease inhibitors and α-amilase inhibitors have been shown to be insecticidal effects towards a range of economically important insect pests by direct assay or by expression in transgenic plants (Ishimoto et al., 1989; Ryan, 1990; Chrispeels et al., 1998; Gatehouse et al., 1998; Ussuf et al., 2001).

So far, one of the efficient strategies against insect pests has been exclusively based on lectins, mainly from the mannose and mannose/glucose. Some of these, such as *Galanthus nivalis* agglutinin (GNA) a mannose specific lectin and *Canavalia ensiformis*, agglutinin (ConA) a mannose/glucose specific lectin have been successfully engineered into a variety of crops including wheat, tobacco, sugarcane, rice and potatoes as resistant factor against some of their insect attacks (Powell et al., 1993; Gatehouse et al., 1996; Sauvion et al., 2004).

More recently, in addition to the use of a broad range of plant lectins, another source of protein derived from fungi was successfully delivered via artificial diet against some of important insect pests, and were shown to have high deleterious effects on these insects, compared to plant lectins (Trigueros et al., 2003). One of the most important features of fungal lectins, compatible with the proposed defensive function, is their high toxic property to aphids' pests, compared to well-known plant lectins (Trigueros et al., 2003; Karimi et al., 2007).

In this review, we attempted to analyze the literature data on the role played by entomotoxic proteins in the very complex relationships between plants and the insects that feed on plants.

2. PLANT-INSECT INTERACTIONS

On one hand, plants and insects are some of the living organisms that are continuously interacting in a complex way. Plants developed different mechanisms to reduce insect attack, including specific responses that activate different metabolic pathways which considerably alter their chemical and physical aspects (Ryan, 1990; Smith et al., 2006). The best-known plant substances supposedly involved in defence mechanisms against phytophagous insects are ribosome-inactivating proteins (RIPs), protease inhibitors (PIs), amylase inhibitors (αAl-I) and lectins. They are particularly abundant in plant storage organs such as tubers and seeds (Ferrari et al., 1991; Aronson, 1994; Peumans et al., 1995; Koiwa et al., 1997; Halitschke et al., 2001; Murdock et al., 2002; Carlini et al., 2002; Bell et al., 2004).

On the other hand, insects have developed several strategies to overcome plant defense barriers, allowing them to feed, grow and reproduce on their host plants. However, insects possess a powerful assemblage of enzymes that constitute their defense against chemical toxicants (Chrispeels et al., 1991; Scott et al., 2001). The better understanding of this complex interaction between plants and insects will allow us to achieve more effective methods for the biological control of insect pests with natural products by developing new plant varieties with enhanced genetically modified (GM) crop (Haruta et al., 2001; Halitschke et al., 2001).

3. ENTOMOTOXIC PROTEINS

So far, extensive studies have been carried out to identify proteins with insecticidal properties against major economic pests. There are many proteins from different origins with direct insecticidal property and ability to express in transgenic plants. Among them some enzyme inhibitors and lectins are being evaluated for their ability to confer the broad-spectrum insect resistance in transgenic crop plants or by direct assays to control of insect pests (Carlini et al., 2002; Vasconcelos et al., 2004).

3.1. Ribosome-inactivating proteins

Ribosome-inactivating proteins (RIPs) are a group of plant proteins that are capable of inactivating eukaryotic ribosome's and accordingly are called ribosome-inactivating proteins, which play an important role in plant defense and hence can be exploited in plant protection (Van Damme et al., 2001; Peumans et al., 2001; Sharma et al., 2004). Several lines of evidence support the idea that RIPs play a role in plant defense (Nielsen et al., 2001; Peumans et al., 2001). RIPs are subdivided on the basis of their molecular structure into three distinct groups. Type-I RIPs is composed of a single polypeptide chain and endowed with a variety of activities including immunosuppressive/anti-mitogenic, anti-tumor/anti-proliferative, and anti-viral activities (Ng et al., 1992; Barbieri et al., 1993; 1996). More recently, Bertholdo-Vargas et al. (2009) reported that type-I RIPs has also entomotoxic activity toward Lepidopteran insects. Type-II RIPs is a heterodimer consisting of two polypeptide chains (A & B chains), which A-chain is linked through a disulfide bridge to a B-chain. The A-chain has an N-glucosidase activity of the ribosomal ribonucleic acid (rRNA) and B-chain contains carbohydrate-binding domains and is also regarded as lectin. They are defence proteins that directly targeted plants eating organisms. Whereas, type-III RIPs has a single chain containing an extended carboxyl-terminal domain with unknown function (Barbieri et al., 1993; 1996; Van Damme et al., 2001; Peumans et al., 2001; Bass et al., 2004).

Ricin, abrin and modeccin are well known examples of RIPs, which irreversibly inactivate ribosomes by removing a specific adenine from a highly conserved tetra-nucleotide loop present in the large ribosomal subunit (Endo et al., 1987; Barbieri et al., 1993). Some of these such as ricin (type-II RIPs) have high toxicity effect against a variety of insects, although these effects are variable on different insect orders (Gatehouse et al., 1990; Wei et al., 2004). More recently, Shahidi-Noghabi et al. (2009) demonstrated that expression of *Sambucus niger* agglutinin (SNA-I, type-II RIPs) from elderberry bark in transgenic tobacco has a deleterious effect on two important insect pests, the tobacco aphid *Myzus nicotianae* and the beet armyworm *Spodoptera exigua*. These information provide further support for RIPs having a role in the plant resistance to insect pest species.

RIPs may be able to bind to specific sites on the cell surfaces; either exerting their toxic action at the membrane level or after uptake and internalization of the toxic polypeptide chain. Most RIPs specifically recognize galactosyl terminated glycoproteins on the cell surface and as such facilitate the entry of the RIPs onto the cell, where it can exert its enzymatic activity on ribosome or other cellular structures. Although the biochemical properties of the RIPs are well studied, but their exact mechanisms of action at the tissue level of RIPs-ingested insects are not well understood (Vervecken et al., 2000; Stirpe et al., 2006; Shahidi-Noghabi et al., 2008; 2009; Bertholdo-Vargas et al., 2009).

Recently, one kind of RIPs from winter aconite, *Eranthis hyemalis* (Ranunculales: Ranunculaceae) has been shown to have high toxicity effect to mammals (Carlini et al., 2002).

3.2. Protease inhibitors

Proteases inhibitors (PIs) are polypeptides that occur in a wide variety of plants, and are able to bind to insects mid-gut proteolytic enzymes, rendering them inactive by competitive inhibition (Laskowski et al., 1980), thus, providing a natural defense against herbivorous insects (Ryan, 1990). In plants, different roles for protease inhibitors have been suggested, including their action as regulators of endogenous proteolytic activity (Ryan, 1990), as participants in many developmental processes, as programmed cell death (Solomon et al., 1999), and as components associated with the resistance of plants against insects and pathogens (Etzler, 1986; Hilder et al., 1987; Lu et al., 1998; Pernas et al., 1999; Gatehouse et al., 1999; Ussuf et al., 2001; Rahbé et al., 2003a). PIs have been shown to function as plant defense molecules and were considered for use in preventing insect predation. They could be good candidate proteins to be used by direct assays or by expression in transgenic plants to the control of some insect pests (Green et al., 1972; Etzler, 1986; Hilder et al., 1987; Koiwa et al., 1998; Mosolov et al., 2001; Srinivasan et al., 2005).

The defensive capacities of plant PIs rely on the inhibition of proteases present in insect guts, causing a reduction in the availability of amino acids necessary for their growth and development (De Leo et al., 1998; 2002; Hilder et al., 1999; Rahbé et al., 2003a; Zhu-Salzman et al., 2003). Insect digestive proteases can be classified as serine, cysteine, aspartic and metallo-proteases inhibitors (Richardson, 1977; Terra et al., 1994). Some of these, such as serine and cysteine proteases inhibitors are the most widely studied, and have been shown that have deleterious effects on two important orders of insect pests including Lepidopteran and Coleopteran, which may include reduced fecundity, increased mortality and decreased weight (Kuroda et al., 1996; Gruden et al., 1998; Murdock et al., 1988; Elden, 2000; Maqbool et al., 2001). A number of Coleopteran insects' mid-gut are slightly acidic and utilize cysteine and aspartic proteases to hydrolyse their dietary proteins, while most of Lepidopteran pests have alkaline mid-gut environment and use serine proteases as their major digestive enzymes (Kitch et al., 1986; Murdock et al., 1987; Terra et al., 1994).

So far, cDNA sequences encoding different protease inhibitors have been expressed in the genome of different important plants such as potato, oilseed, rapeseed, tobacco, cereals and protective effects have been obtained in some cases, mainly against Lepidopteran, Coleopteran and Hemipteran pests (Leple et al., 1995; Urwin et al., 1995, 1998; Koiwa et al., 1997; 1998; Schuler et al., 1998; Lecardonnel et al., 1999; Ussuf et al., 2001; Maqbool et al., 2001; Ceci et al., 2003; Macedo et al., 2003; Rahbé et al., 2003a; 2003b). PIs have been used to induce enhance level of resistance to insect pests in transgenic plants, due to their small size, abundance, stability and high specificity for a particular class of digestive enzymes of insects (Ussuf et al., 2001; Abdeen et al., 2005).

The greatest interest will probably be attracted to plants containing a combination of genes encoding protease inhibitors and other protective proteins. For example, the effect of Bt toxin was synergized by protease inhibitors. In such cases, the inhibitors can probably act not only as self-sufficient protective proteins, but can also protect other recombinant proteins from the deleterious effects of proteolytic enzymes (De Leo et al., 1998; Cloutier et al., 2000; Mosolov et al., 2001; Zhao et al., 2003; Christou et al., 2006).

The action mode of PIs at the tissue level of PIs-ingestion insect are extremely selective and different types of those have different action mechanism and biological processes. Ramos et al. (2009) suggested that the toxic effect of the protease inhibitors induced the insect to eliminate its digestive enzymes in feces, complicating its digestion. In contrast, some insects such as *Spodoptera littoralis* (Lepidopteran), can overcome the deleterious effects of protease inhibitors by synthesizing different proteases that are insensitive to particular inhibitors (Paulillo et al., 2000; Brito et al., 2001; De Leo et al., 2001; Volpicella et al., 2003). Whereas, the exact action mechanism of PIs at the tissue level of insects is not well-known (Jongsma et al., 1997; Carlini et al., 2002; Amirhusin et al., 2007).

3.3. α-Amylase inhibitors

Alpha-amylase inhibitors (αAl-I) are known as starch blockers because they contain substances that prevent dietary starches from being absorbed by the body. Microorganisms, higher plants, and animals produce a large number of different protein inhibitors of α-amylases in order to regulate the activity of these enzymes (Da Silva et al., 2000; Toledo et al., 2007). These inhibitors widely distributed in the most important digestive enzymes of many insects that feed exclusively on seed products during larval and/or adult life, thereby acting as insect anti-feedants (Chrispeels et al., 1991). Alpha-amylase inhibitors can be extracted from several types of plants, especially those in the legume family and also use as a defence mechanism against insect pests (Ishimoto et al., 1989; Kluh et al., 2005). In addition, amylase inhibitors are of great interest as potentially important tool of natural and engineered resistance factor against some of insect pests in transgenic plants (Gatehouse et al., 1998; Valencia et al., 2000; Yamada et al., 2001). The expression of the cDNA encoding αAl-I into some plants such as pea (*Pisum sativum* L.) and azuki bean (*Vigna anguralis* L.) against bruchid beetle pests (Coleoptera: Bruchidae) has been well documented for showing ability of these inhibitors to be used as plant resistance factors against some species of insect pests (Ishimoto et al., 1989; Yamada et al., 2001; Kluh et al., 2005).

3.4. Lectins

Lectins are a class of proteins of non-immune origin that possess at least one non-catalytic domain that specifically and reversibly bind to mono- or oligosaccharides (Dixon, 1981; Peumans et al., 1995). They encompass different members that are diverse in their sequences, structures, binding site architectures, carbohydrate affinities, and specificities as well as their larger biological roles and potential applications. However, today the term lectin is broadly used to denote all types of carbohydrate-binding proteins that do not catalyse reactions with their ligands (Peumans et al., 1995; Van Damme et al., 1998; Chandra et al., 2006). Lectins bind mono- and oligosaccharides reversibly with high specificity, defined in terms of the monosaccharide that inhibit lectin-induced agglutination or precipitation reactions. These glycoproteins are multivalent and possess more than one sugar binding site (Lis et al., 1998). They firstly were discovered more than 100 years ago by Stillmark (1888) and currently, they are extensively distributed in nature and several hundred of these molecules have been isolated from different organisms (Peumans et al., 1995; Van Damme et al., 1998). Different roles and functions have been ascribed to lectins. The principal functions of lectins are to act as recognition molecules within the immune system, protein storage, cell surface adhesion and they have been implicated in defence mechanisms against invading pathogens and predators, this function may be a major role within seeds and other peripheral tissues of the plants (Peumans et al., 1995; Van Damme et al., 1998; Rudiger et al., 2001; Trigueros et al., 2003). Lectins are broadly defined as carbohydrate-binding proteins other than enzymes or antibodies with the characteristic property of agglutinating blood cells (Van Damme et al., 1998).

Various lectins have already been found to be toxic towards important members of insect orders, including Coleoptera (Gatehouse et al., 1984; Czapla et al., 1990), Lepidoptera (Czapla et al., 1990) and Homoptera (Powell et al., 1993; Sauvion et al., 1996). The harmful effects of lectins on 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). Recently, a wide range of lectins from different organisms have been successfully examined for their negative effects on the life parameters of some economically insect pests (Foissac et al., 2000; Couty et al., 2001; Trigueros et al., 2003; Sauvion et al., 2004; Karimi et al., 2007; Shahidi-Noghabi et al., 2008; 2009).

Sources of lectins. Lectins are extensively distributed in nature and several hundred of these molecules have been isolated from plants, fungi, viruses, bacteria, invertebrates and vertebrates (Peumans et al., 1995). Recently, one of the promising methods for plant resistance against insects attacks have increased the interest in the potential toxicity of plant and fungal lectins towards some of important insect pests (Foissac et al., 2000; Carlini et al., 2002; Trigueros et al., 2003; Sauvion et al., 2004; Karimi et al., 2007).

Plant lectins. The best-characterized families of plant lectins are the Fabaceae, Poaceae and Solanaceae; especially some leguminous seeds have a remarkable amount of lectins. Lectins have been found in virtually, all kinds of vegetative tissues such as leaf, stem, bark, bulb, tuber, corm, rhizome, root, fruit, flower, ovary, phloem sap, latex, nodule, seed, stem and even in nectar (Van Damme et al., 1998). Plant lectins function as storage proteins and they have been implicated in defence mechanisms against invading pathogens (Powell et al., 1993; Peumans et al., 1995; Van Damme et al., 1998; Rudiger et al., 2001). The insecticidal activity of plant lectins against many important insects has been well documented to show their ability to be used as bio-pesticides (**Table 1**) (Gatehouse et al., 1995; Powell, 2001; Carlini et al., 2002). Among plant lectins presented in **table 1** as entomotoxic lectins, GNA and ConA were more investigated and delivered successfully, via artificial diet and expressed in a range of crops, they have been shown to present deleterious effects on a range of important insect pests (Down et al., 1996; Fitches etal., 1997; 2001b; Rao et al., 1998; Foissac et al., 2000).

GNA have been expressed in various crops including potato (Brich et al., 1999), tobacco (Hilder et al., 1987), wheat (Stoger et al., 1999), rice (Rao et al., 1998), sugarcane (Allsopp et al., 1996) and have been shown to exhibit significant anti-insect effects against a number of insect orders, including Lepidopteran (Fitches et al., 1997; Bell et al., 1999), Coleopteran (Leple et al., 1995) and Homopteran (Down et al., 1996; Gatehouse et al., 1996).

ConA has shown also deleterious effect on some insect orders such as Homoptera (Powell, 2001) and Lepidoptera, when expressed in transgenic plants (Gatehouse et al., 1999). ConA affected survival; delayed development durations, larval weight and mortality of these orders that have ingested them. The effect of ConA consumption on these orders may fluctuate among different species because, some species are highly susceptible to ConA, and the effect on other may be less or moderate (Rahbé et al., 1993; Gatehouse et al., 1997; 1999; Sauvion et al., 2004).

Fungal lectins. Mushrooms contain various potential interesting proteins, including lectins in their organs such as mycelium, spores and fruiting bodies (Wang et al., 1998; Wang et al., 2002; Ng, 2004). Many lectins have been derived from different fungi and partially isolated and characterized for their effects on mammalian physiology as antitumor and anticancer, but there are little information on their role on phyto-phagous insects (Wang et al., 2002; Trigueros et al., 2003). Until now, a range of mushroom lectins including *Xerocomus chrysenteron* lectin (XCL), *Agaricus bisprous* lectin (ABL) and *Arthrobotrys oligospora* lectin (AOL) have been isolated and all of them are well known for their reversible antiproliferative effects. But, only XCL has well shown significant negative effects on some insect orders, such as the Dipteran (*Drosophila melanogaster*) and Hemipteran (*Acyrthosipon pisum*), and exhibited a higher insecticidal activity than the GNA, which is one of the most lectins toxic to these insects (Trigueros et al., 2003). XCL incorporated with artificial diets has been shown highly toxic effect toward two important Homopteran pests, *Myzus persicae* and *A. pisum* when compared with control lectin, ConA (Karimi et al., 2006; 2007). XCL is produced by an edible mushroom and belongs to a group of lectins which first described for AOL, a Deuteromycetes and ABL, a Basidiomycete for sequence homology and sugar specificity, which are specific for N-acetyl-D-galactosamine and galactose (Trigueros et al., 2003; Francis et al., 2003). Recently, the entomotoxic proteins based on fungal lectin appear to be a promising biological control agent against some of the important insect pests.

Lectin mechanism of actions. Entomotoxic lectins either directly or indirectly cause profound morphological and physiological modifications in the insect intestine. The investigations on possible mechanisms of lectin toxicity in the insects at the cellular level were initiated 24 years ago, when Gatehouse et al. (1984) firstly reported the binding of *Phaseolus vulgaris* lectin (PHA) to midgut epithelial cells of the cowpea weevil, *Callosobruchus maculatus*.

In fact, the mode of action for each lectin at the tissue level is related to the occurrence of appropriate carbohydrate moieties on the organ surface and the ability of lectin to bind to them (Fitches et al., 2001a; 2001b). In general, the action mechanism of the lectin in insects could be the binding of them 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 (Gatehouse et al., 1984; Powell et al., 1998; Habibi et al., 1998; 2000; Fitches et al., 1998; 2001b; Sauvion et al., 2004; Majumder et al., 2005).

Table 1. Plant lectins with oral toxicity to insects — *Lectines végétales ayant une toxicité orale pour les insectes* (Vasconcelos et al., 2004).

Lectin (plant source)	**Insects**	**Hosts**	**References**
Mannose specific			
ASA (*Allium sativum*)	*Laodelpha striatellus* (rice small brown planthopper); *Nilaparvata lugens* (rice brown planthopper);	Rice	Powell et al., 1995
	Myzus persicae (peach-potato aphid)	Peach, potato	Sauvion et al., 1996
	Dysdercus cingulatus (red cotton bug); *Dysdercus koenigii* (red cotton bug)	Cotton, okra, maize, pearl	Roy et al., 2002
ASA I, II	*Dysdercus cingulatus*; *Dysdercus koenigii*	Cotton, okra, maize, millet	Roy et al., 2002
ASAL (*Allium sativum*, leaf)	*Dysdercus cingulatus*; *Lipaphis erysimi* (mustard aphid)	Cotton, okra, maize, pearl	Bandyopadhyay et al., 2001
CEA (*Colocasia esculenta*)	*Dysdercus cingulatus*; *Dysdercus koenigii*	Cotton, okra, maize, pearl	Roy et al., 2002
DEA(*Differenbachia sequina*)	*Dysdercus cingulatus*; *Dysdercus koenigii*	Cotton, okra, maize, pearl	Roy et al., 2002
GNA (*Galanthus nivalis*)	*Callosobruchus maculatus* (bruchid weevil)	Cowpea	Gatehouse et al., 1991
	Acyrthosiphon pisum (pea aphid)	Pea	Rahbé et al., 1995
	Antitrogus sanguineus (sugarcane whitegrub)	Sugarcane	Allsopp et al., 1996
	Aulacorthum solani (glasshouse potato aphid)	Potato	Down et al., 1996
	Myzus persicae	Peach, potato	Sauvion et al., 1996
	Lacanobia oleracea (tomato moth)	Tomato	Fitches et al., 1998; 2001a
	Maruca vitrata (legume pod-bore)	Cowpea	Machuka et al., 1999
	Tarophagous proserpina (taro planthopper)	Taro	Powell, 2001
	Laodelpha striatellus	Rice	Loc et al., 2002
	Nilaparvata lugens	Rice	Powell et al., 1995; 1998; Loc et al., 2002
KPA (*Koelreuteria paniculata*)	*Anagasta kuehniella* (Mediterranean flour moth); *Callosobruchus maculatus*	Beans, grains, fruits, nuts	Macedo et al., 2003
LOA (*Listera ovata*)	*Maruca vitrata*	Cowpea	Machuka et al., 1999
NPA (*Narcissus pseudonarcissus*)	*Nilaparvata lugens*; *Myzus persicae*	Rice Peach, potato	Powell et al., 1995 Sauvion et al., 1996
Mannose/glucose specific			
ConA(*Canavalia ensiformis*)	*Acyrthosiphon pisum*	Pea	Rahbé et al., 1993
	Acyrthosiphon pisum	Pea	Rahbé et al., 1995
	Aphis gossypii (cotton and melon aphid)	Cotton, mellon	Rahbé et al., 1995
	Aulacorthum solani (glasshouse and potato aphid)	Potato	Rahbé et al., 1995
	Macrosiphum albifrons (lupin aphid)	Lupin	Rahbé et al., 1995
	Macrosiphum euphorbiae (potato aphid)	Apple, bean, broccoli, papaya	Rahbé et al., 1995
	Myzus persicae	Peach, potato	Rahbé et al., 1995; Sauvion et al., 1996; Gatehouse et al., 1999
	Lacanobia oleracea	Tomato	Fitches et al., 1998; Gatehouse et al., 1999; Fitches et al., 2001a
	Tarophagous proserpina	Taro	Powell, 2001
LCA (*Lens culinaris*)	*Acyrthosiphon pisum*	Pea	Rahbé et al., 1995

Table 1 (continued). Plant lectins with oral toxicity to insects — *Lectines végétales ayant une toxicité orale pour les insectes* (Vasconcelos et al., 2004).

Lectin (plant source)	Insects	Hosts	References
PSA (*Pisum sativum*)	*Acyrthosiphon pisum*	Pea	Rahbé et al., 1995
	Hypera postica (clover leaf weevil)	Alfafa, lucerne	Elden, 2000
N-acetyl-D-glucosamine specific			
ACA (*Amaranthus caudatus*)	*Acyrthosiphon pisum*	Pea	Rahbé et al., 1995
BSA (*Bandeiraea simplicifolia*)	*Diabrotica undecimpunctata* (Southern corn rootworm); *Ostrinia nubilaris* (European corn borer)	Corn	Czapla et al., 1990
BSAII	*Acyrthosiphon pisum*	Pea	Rahbé et al., 1995
GSII (*Griffonia simplicifolia*)	*Callosobruchus maculatus*	Cowpea	Zhu et al., 1996; Zhu-Salzman et al., 1998; 2001
PAA (*Phytolacca americana*)	*Diabrotica undecimpunctata*; *Ostrinia nubilaris*	Corn	Czapla et al., 1990
TEL (*Talisia esculenta*)	*Callosobruchus maculatus*; *Zabrotes subfasciatus* (Mexican dry bean weevil)	Beans	Macedo et al., 2002
WGA (*Triticum aestivum*)	*Diabrotica undecimpunctata*; *Ostrinia nubilaris*	Corn	Czapla et al., 1990 Allsopp et al., 1996
	Antitrogus sanguineus (sugarcane whitegrub)	Sugarcane	Elden, 2000
	Hypera postica	Alfafa	Kanrar et al., 2002
	Lipaphis erysimi	Mustard	
Galactose specific			
AHA (*Artocarpus hirsuta*)	*Tribolium castaneum* (red flour beetle)	Large number of grains	Gurjar et al., 2000
AIA (*Artocarpus integrifolia*)	*Diabrotica undecimpunctata*; *Ostrinia nubilaris*	Corn	Czapla et al., 1990
GHA (*Glechoma hederacea*, leaf)	*Leptinotorsa decemlineata* (colorado potato beetle)	Potato	Wang et al., 2003
RCA120 (*Ricinus communis*)	*Diabrotica undecimpunctata*; *Ostrinia nubilaris*	Corn	Czapla et al., 1990
YBA (*Sphenostylis stenocarpa*)	*Clavigralla tomentosicollis* (coreid bug)	Vigna spp.	Okeola et al., 2001
	Callosobruchus maculatus; *Maruca vitrata*	Cowpea	Machuka et al., 2000
N-acetyl-D-galactosamine specific			
ACA (*Amaranthus caudatus*)	*Acyrthosiphon pisum*	Pea	Rahbé et al., 1995
BFA (*Brassica fructiculosa*)	*Brevicoryne brassicae* (cabbage aphid)	Broccoli, Brussels sprouts, cauliflower, head cabbage	Cole, 1994
BPA (*Bauhinia purpurea*)	*Diabrotica undecimpunctata*; *Ostrinia nubilaris*	Corn	Czapla et al., 1990
CFA (*Codium fragile*)	*Diabrotica undecimpunctata*; *Ostrinia nubilaris*	Corn	Czapla et al., 1990
EHA (*Eranthis hyemalis*)	*Diabrotica undecimpunctata*	Corn	Kumar et al., 1993
MPA (*Maclura pomifera*)	*Diabrotica undecimpunctata*; *Ostrinia nubilaris*	Corn	Czapla et al., 1990
PTA (*Psophocarpus tetragonolobus*)	*Callosobruchus maculatus* *Nilaparvata lugens*	Cowpea Rice	Gatehouse et al., 1991 Powell, 2001
SNA-II (*Sambucus nigra*)	*Acyrthosiphon pisum*	Pea	Rahbé et al., 1995
VVA	*Diabrotica undecimpunctata*; *Ostrinia nubilaris*	Corn	Czapla et al., 1990
PHA (*Phaseolus vulgaris*)	*Lygus hesperus* (Western tarnished plant bug)	Cotton, alfafa, legumes	Habibi et al., 2000

Consequently, the action mechanism of various lectins at the cellular levels clearly differs between different insect species, which are highly specific binding to oligosaccharides (Habibi et al., 2000; Fitches et al., 2001a; Sauvion et al., 2004).

Lectin interaction in virus transmission. Plant viruses may be transmitted by number of routes including: seeds, vegetative propagation/grafting, mechanical and vectors. Many different groups of living organisms such as bacteria, fungi, nematodes, mites and insects can act as vectors and spread viruses from one plant to another (Gray, 1996). Currently, many of important plant viruses are transmitted by insects of the Hemiptera order (sap-sucking insects) including aphids, whiteflies, leafhoppers, planthoppers, and thrips (Gildow, 1993; Gray, 1996; Gray et al., 2003).

Plant viruses transmitted by insects are divided into two broad categories with different transmission processes: circulative and non circulative viruses. Circulative viruses are usually defined as moving from the alimentary canal of a vector insect onto its hemocoel and are able to back out through the salivary secretory system, from which these viruses are introduced back into the plant host during insect feeding (**Figure 1**). Whereas, non circulative virus associated with the cuticular lining of the insect mouthparts or foregut and directly released as digestive secretions onto the plant when insect begins to feed (Gray, 1996; Gray et al., 1999; 2003; Hogenhout et al., 2008).

The initiation of a successful virus infection cycle requires the attachment of virions to specific receptor molecules on the surfaces of host cells, with subsequent penetration of and entry into the host cells for the release of viral genomes for replication (Dimitrov, 2004; Marsh et al., 2006). Some electron microscopic observations have suggested that plant viruses enter their vector insect cells via receptor-mediated endocytosis; for example, luteoviruses were believed to move across the alimentary canal to hemocoel and salivary glands of aphid vectors by endocytosis phenomena (Garret et al., 1993; Gray et al., 2003). Electron microscopy, immunofluorescence studies, and experiments with various inhibitors support the hypothesis that Rice Dwarf Virus (RDV) enters vector insect cells through receptor-mediated, clathrin-dependent endocytosis and is sequestered in a low-pH-dependent endosomal compartment (Wei et al., 2007).

Understanding the mechanisms of virus transmission is the key to developing effective strategies to block virus-vector interactions. The principal mechanisms of recognition of cells, proteins, tissues or signals involve the recognition of carbohydrates residues (Pereira et al., 2008). Some lectins are able to identify and bind various pathogens, including viral glycoproteins. By the recognition and the binding with viral glycoproteins, lectins are able to decrease the binding of virus with receptors (Thielens et al., 2002; Naidu et al., 2004) and subsequently, avoid the transport of virus from gut to hemocoel of insect vector (Nelson et al., 2005; Desoignies, 2008). Finally, in this case virus transmission will be suspended (**Figure 1**).

However, Desoignies (2008) reported that transmission of Potato virus Y (PVY) through *Myzus persicae* was significantly reduced when this aphid fed on an artificial diet incorporating *Pisum sativum* lectin (PSL).

Lectin applications

Plants producing natural lectin. In general the plants that are surrounding the main crops are the first barrier to insect attacks. There are many plants such as cowpea, broad and green beans, pea that are able to produce natural lectins in their phloem (Van Damme et al., 1998). These plants are compatible as an intercrop with many crop plants. Therefore, the use of these plants surrounding the main crops is one of the potential ways to reduce insect damage and the virus transmission. In fact, when the insects feed on these plants which have injected lectin, they would not more be able to act as vectors of virus. Indeed, transmission of virus by these insects will decrease because of their receptor site has been blocked by lectin and virus is not able to pass the alimentary canal to reach onto salivary canal and injection again in plants (Gray, 1996; Gray et al., 2003; Desoignies, 2008).

Gene isolation and transgenic plant proteins. Transgenic plants technology or genetically modified (GM) crops can be a useful tool in producing resistant crops, by introducing novel resistance genes into plants provides a sustainable alternative to the control of insect pests and phytovirus (Gatehouse et al., 1997; 1999; Gray et al., 2003). Currently, the two major groups of plant-derived genes used to confer insect resistance on crops are those of inhibitors of digestive enzymes (proteases and amylase inhibitors) and lectins have been introduced into crops genomes and are now being tested in field conditions (Gatehouse et al., 1993; Hilder et al., 1987; Hilder et al., 1999; Carlini et al., 2002; Schuler et al., 1998). Additionally, crops have been engineered to express a range of insect-plant resistance (**Table 2**), and have been shown to confer enhanced levels of resistance to different order insect pests including Lepidopteran (Gatehouse et al., 1997), and Homopteran (Down et al., 1996; Gatehouse et al., 1996), when expressed in wheat (Stoger et al., 1999), and rice (Rao et al., 1998) resulting in increased resistance to aphids and plant hoppers, respectively (Powell et al., 1998).

Until now, many lectins from different origins such as *Allium sativum* agglutinin (ASA), Wheat germ agglutinin (WGA), *Urtica dioica* agglutinin (UDA),

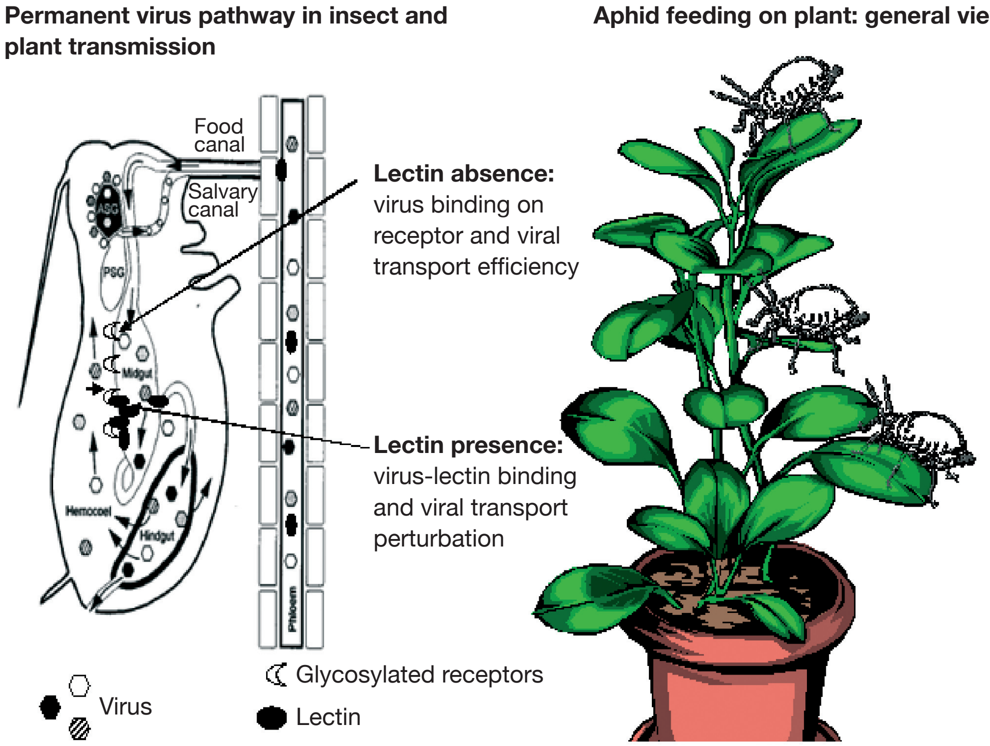

Figure 1. Schematic representation of interaction transmission of virus by aphids feeding on plant tissues (figure adapted from Gray et al., 1999) — *Dessin schématisant l'interaction de transmission du virus par les pucerons se nourrissant des tissus végétaux (Figure adaptée de Gray et al., 1999).*

Circulative viruses strains can be ingested from plant phloem into the aphids alimentary canal and arrive in the hindgut intact. The hindgut epithelium is the first transmission barrier; most circulative viruses can bind to receptors on the surface of hindgut and occasionally to the midgut epithelial cells and are able to be transported into the hemocoel. Virus acquired in the hemocoel must migrate to the accessory salivary glands (ASG). Here, virus is actively transported across two distinct barriers to transmission, the ASG basal lamina and the ASG plasmalemma and then released into the salivary canal (SC). Finally, virus is injected by means of SC into a plant as the aphid feeds. The basal lamina of the ASG may selectively filter virus or may concentrate virions, thereby increasing the efficiency of transport into the ASG and the salivary canal. But, when aphids fed on transgenic plants (gene encoding lectin), there is interaction between lectin and envelope membrane glycoproteins of virus in alimentary canal of this aphid. In this case lectin will be able to bind with oligosaccharides present on the virus envelope glycoproteins and virus transmission will be perturbated — *Les souches de virus circulants peuvent être ingérées au niveau du phloème de la plante au travers du tube digestif des pucerons et arriver dans l'intestin postérieur. L'épithélium de l'intestin postérieur est la première barrière à la transmission ; la plupart des virus circulants peuvent se lier à des récepteurs à la surface de l'intestin postérieur et occasionnellement aux cellules épithéliales de l'intestin moyen et sont capables d'être transportés dans l'hémocèle. Les virus acquis dans l'hémocèle doivent migrer vers les glandes salivaires accessoires (ASG). Le virus est activement transporté à travers deux barrières à la transmission, la lame basale des ASG et les plasmalemmes des ASG, puis libéré dans le canal salivaire (SC). Enfin, le virus est injecté dans la plante par le canal salivaire lorsque le puceron se nourrit. La lame basale de l'ASG filtrerait sélectivement le virus ou concentrerait les virions, ce qui augmente l'efficacité du transport dans les ASG et le canal salivaire. Lorsque les pucerons se nourrissent de plantes transgéniques (gène codant de lectine), il existe une interaction entre la lectine et des glycoprotéines du virus dans le canal alimentaire de ce puceron. Dans ce cas, la lectine sera capable de se lier à des oligosaccharides présents sur les glycoprotéines d'enveloppe du virus et finalement la transmission du virus sera perturbée.*

Phytohemagglutinin (PHA), a lectin from the red kidney bean, *Phaseolus vulgaris* L., *Pisum sativum* lectin (PSL), ConA, XCL and GNA have been isolated and partially characterized for their effects on insect pests, but among them some of encoding insecticidal lectins such as GNA, ConA, WGA and PSA have been shown greater potential for expression into a variety of crops including wheat, tobacco, sugarcane, rice and potatoes as resistant factors against some of important insect pests (Maddock et al., 1991; Powell et al., 1993; 1995; Habibi et al., 1993; Hilder et al., 1995; Gatehouse et al., 1996; 1997; 1999; Down et al., 1996; Fitches et al., 1997; Rao et al., 1998; Kanrar et al., 2002; Setamou et al., 2002) (**Table 2**).

Overall, transgenic plants expressing high levels of lectins exhibited some degree of resistance to the target insects. It is worth mentioning that the great interest on transgenic crop plants expressing the gene for GNA, ConA and WGA or other mannose-binding lectins such as ASA resides in the fact that although these lectins

Table 2. Plant species transformed with lectin genes to confer resistance against the insects — *Espèces de plantes transformées grâce à des gènes de lectine pour conférer une résistance aux insectes* (Vasconcelos et al., 2004).

Transformed plant	Lectin	Target pests	References
Maize	WGA	*Ostrinia nubilaris*; *Diabrotica undecimpunctata*	Maddock et al., 1991
Mustard (*Brassica juncea*)	WGA	*Lipaphis erysimi*	Kanrar et al., 2002
Arabidopsis thaliana	PHA-E, Lb	*Lacanobia oleracea*	Fitches et al., 2001b
Potato	GNA	*Aulacorthum solani*	Down et al., 1996
Potato	GNA	*Myzus persicae*	Gatehouse et al., 1996; Couty et al., 2001
Potato	GNA	*Lacanobia oleracea*	Fitches et al., 1997; Gatehouse et al., 1997
Potato	GNA	*Lacanobia oleracea*	Bell et al., 1999; 2001; Down et al., 2001
Potato	GNA	*Aphidius ervi* (parasitoid of *Myzus persicae*)	Couty et al., 2001
Potato	ConA	*Lacanobia oleracea*; *Myzus persicae*	Gatehouse et al., 1999
Rice	GNA	*Nilaparvata lugens*	Rao et al., 1998; Foissac et al., 2000; Tinjuangjun et al., 2000; Maqbool et al., 2001; Tang et al., 2001; Loc et al., 2002
Rice	GNA	*Nephotettix virescens* (green leafhopper)	Foissac et al., 2000
Rice	GNA	*Cnaphalocrocis medinalis* (rice leaffolder); *Scirpophaga incertulas* (yellow stemborer)	Maqbool et al., 2001
Rice	GNA	*Laodelphax striatellus* (rice small brown planthopper)	Sun et al., 2002; Wu et al., 2002
Sugarcane	GNA	*Eoreuma loftini* (Mexican rice borer); *Diatraea saccharalis* (sugarcane borer)	Setamou et al., 2002
Sugarcane	GNA	*Parallorhogas pyralophagus* (parasitoid of *Eoreuma loftini*)	Tomov et al., 2003
Tobacco	PSA	*Heliothis virescens* (tobacco budworm)	Boulter et al., 1990*
Tobacco	GNA	*Myzus persicae*	Hilder et al., 1995
Tobacco	GNA	*Helicoverpa zea* (cotton bollworm)	Wang et al., 1999
Wheat	GNA	*Sitobion avenae* (grain aphid)	Stoger et al., 1999

* First demonstration of insect enhanced resistance of transgenic plants expressing a foreign lectin.

show toxicity against species of various insect orders, they are non toxic to mammals and birds (Powell et al., 1995; Down et al., 1996; Bandyopadhyay et al., 2001). Therefore GNA, ConA and WGA have been transferred and expressed in several crop plants (**Table 2**). Genetic engineering of crop plants has been developed as an alternative to chemical insecticides for plant protections against insect pests (Ranjekar et al., 2003).

Synergistic effects on other proteins. One of the interest applications of lectin is its synergistic effect on the other entomotoxic proteins. In fact, most resistance phenomena of insects to toxic protein such as Bt toxin is due to their unique resistance gene (Cao et al., 2002). Therefore, expression of multiple resistance genes such as combination of Bt toxin and lectin gene in plants, can increase their resistance to insect pests. Maqbool et al. (2001) reported that rice plants carrying three insecticidal genes including cry1Ac, cry2A and the snowdrop lectin gene (encoding gene GNA) showed enhanced level of resistance to a range of different rice pests. According to Abdeen et al. (2005) and Amirhusin et al. (2004), anti-insect activities of inhibitor enzymes (protease & α-amilase inhibitors) were often increased, when incorporated with lectin compared with their individual effect. Therefore, lectins are able to use as control agents againts insect pests and they have synergistic effects on other entomotoxic proteins.

4. CONCLUSION

The aim of this literature review was to highlight the ability of some proteins including lectins as resistant factors against some important insect pests to reduce the use of massive chemical compounds. They have been demonstrated direct insecticidal activity to a wide range of insect pests and have a potential to express in transgenic crops to conferring insect-resistant plants. Currently, one of the promising methods for insect-resistance plants has increased the interest on potential toxicity of plant lectins towards some important insect pests, which targeted Homopteran insects which are not affected by Bt toxin and other protein inhibitors with enzymatic activity. In addition to the extensive use of plant lectins, other sources of lectins from fungi including XCL have been presented to control some insect pests. This fungal lectin has been shown with high toxicity effects on some insect pests, compared to plant lectins (ConA & GNA).

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