Chapter 10
Insect and Nematode Resistance

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10.1 Introduction

Crops are attacked by animal pests and nematodes, causing considerable economic losses worldwide. The global yield loss, e.g. due to herbivorous insects varies between 5% and 30% depending on the crop species, while the estimated worldwide losses due to plant parasitic nematodes are about US $125 billion annually (Chitwood 2003). Root-knot nematodes like *Meloidogyne incognita* infect thousands of plant species, resulting in poor fruit yield, stunted growth, wilting and susceptibility to other pathogens. Factors which increase plant susceptibility to pest attacks include a lack of genetic diversity within the genomes of cultivated crop species and changes in cultivation techniques, such as large-scale cropping of genetically uniform plants and reduced crop rotation as well as the expansion of crops into less suitable regions. Use of natural resistance is a promising alternative for parasite control. Advanced understandings of natural resistance mechanisms in molecular details will broaden the horizon of crop resistance breeding programs. As resistance is often limited in many crop species and can be easily overcome by new virulent pathotypes, new genetic variability is therefore needed. Here we give an overview about recent progresses in research of plant resistance genes and the underlying molecular mechanisms as well as their potential in practice application. Today, chemical control of plant parasites depends on relatively few chemicals. These pose serious concerns of risks and hazards for humans, animals and the environment and increase the costs of growing crops. The worldwide use of pesticides increased dramatically since the early 1960s. For example, the synthetic chemical pesticides-based insecticide market is estimated at above US $8 billion

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annually. However, restrictions and especially detriments of pesticide application for pest control (e.g. limited efficiency, inducing resistance of parasites) ask for alternative strategies to ensure a sustainable pest management in agriculture. Consequently, engineered resistance is an essential part of a sustainable parasite control and is becoming more and more important, as it offers a parasite management with benefits to the producer, the consumer and the environment. In this review we focus on the strategy for engineering parasite resistance in crops with anti-parasite genes. Genes are expressed in transgenic plants (for methods, see Chaps. 1, 2) whose products are non-phytotoxic but strongly anti-parasite, either lethally toxic or interfering with parasites after their take-up by parasites, consequently affecting their development and reproduction. Furthermore recent progress in the plant delivery of a RNAi-based gene-silencing strategy (see Chap. 5) provides new tools for engineering broad parasite resistance in crops.

10.2 R Gene-Mediated Resistance

10.2.1 Plant Resistance and Resistance Gene

The use of plant natural resistance mechanisms represents one of the most promising alternatives. Plants have evolved sophisticated and multi-faceted defense mechanisms. Briefly, two branches of the plant immune system exist. The older one, basal immunity (reminiscent of innate immunity in vertebrates), is triggered by pathogen-associated or microbe-associated molecular patterns (PAMP- or MAMP-triggered immunity, PTI); and the second one, effector-triggered immunity (ETI), relies on resistance (R) proteins. Once the pathogen succeeds in suppressing the insufficient basal defenses, plants evolve resistance (R) proteins which directly or indirectly interact in a specific manner with microbial effector proteins and thereby trigger plant immune responses. This is synonymous to pathogen race-plant cultivar-specific host resistance or gene-for-gene resistance (Jones and Takemoto 2004; Jones and Dangl 2006). The recognized effector is termed an avirulence (Avr) protein. Pathogens evolve further and suppress ETI, which again results in new R gene specificities so that ETI can be triggered again (Jones and Takemoto 2004; Jones and Dangl 2006).

To date, numerous R genes have been cloned which confer resistance to several classes of pathogens, including viruses, bacteria, fungi, oomycetes, insects and nematodes. R gene products can be categorized into two main classes based on conserved structural features (Dangl and Jones 2001; Chisholm et al. 2006). The largest class of R proteins (called the NBS-LRR class of R proteins) possesses in addition to a leucine-rich repeat (LRR), a central nucleotide-binding site (NBS) domain. The second major class of R genes encodes extracellular LRR (eLRR) proteins. Three subclasses of LRRs have been suggested according to their domain structures (Fritz-Laylin et al. 2005). These subclasses include receptor-like proteins

(RLP; extracellular LRR and a transmembrane (TM) domain), RLK (extracellular LRR, TM domain, cytoplasmic kinase) and polygalacturonase inhibiting protein (PGIP; cell wall LRR).

Immense progress in plant genome analysis revealed that many R genes are located in clusters that comprise several copies of homologous R gene sequences arising from a single gene family (simple clusters) or colocalized R gene sequences derived from two or more unrelated families (complex clusters). The lack of substantial evidence for direct Avr-R interaction led to the 'guard hypothesis' (Van der Biezen and Jones 1998), which proposes that the X induces a change in a host protein that is normally recruited by the pathogen via its Avr protein to establish a successful infection, and that this change sensed by the R-protein (guard) leads to the activation of the R protein and subsequent defense signaling (Dangl and Jones 2001; Bent and Mackey 2007; van der Hoorn 2008). This model may provide a good explanation for resistance response triggered by other resistance genes.

10.2.2 Plant Parasite Resistance and Resistance Genes

During evolution, different forms of natural resistance to parasites have been established. Plant innate plant defense mechanisms like morphological barriers, diverse compounds of the secondary metabolism and induced resistance mechanisms (PTI) allow only a selected number of parasitic pests to attack a specific range of plant species (Schuler 1998). Often active plant defense is induced immediately after insect attack, leading to the production of various anti-insect compounds, including anti-feedants, toxins and digestibility reducers (Korth 2003; Voelckel and Baldwin 2004a, b). Also indirect defense mechanisms are activated that recruit natural enemies from the plant's surroundings to attack feeding insects (Turlings and Tumlinson 1992; De Moraes et al. 1998; Kessler and Baldwin 2001).

Insect resistance loci have been reported in crop plants like wheat, barley, maize, potato and rice (Yencho et al. 2000). So far, little is known about the underlying molecular mechanisms as the majority of insect resistance loci are mapped as QTLs, making the characterization and the use of these resistance traits for plant breeding difficult and time-consuming. The only cloned insect resistance gene is *Mi-1*. *Mi*, originally isolated as a root knot nematode (*Meloidogyne* spp.) resistance gene from wild tomato (*Lycopersicon peruvianum*) also confers resistance against potato aphids (*Macrosiphum euphorbiae*) and whiteflies (*Bemisia tabaci*; Vos et al. 1998; Martinez de Ilarduya et al. 2001; Nombela et al. 2003).

In contrast, a set of nematode resistance genes have been identified from various crop plants. Economically the most important plant-parasitic nematodes are cyst nematodes of the genus *Heterodera* and *Globodera* and root-knot nematodes of the genus *Meloidogyne*. Root-knoot nematodes of *Meloidogyne* spp. are obligate sedentary endoparasites. Agronomically important species of cyst nematodes, mainly active in temperate regions of the world, are *G. rostochiensis* and *G. pallida*

on potato and *H. glycines* on soybean. In addition, more than 80% of the Chenopodiaceae and Brassicaceae species are hosts of *H. schachtii* (Steele 1965), including economically important crops like sugar beet (*Beta vulgaris*), spinach (*Spinacea oleracea*), radish (*Raphanus sativus*) and rape seed (*Brassica napus*). Today *H. schachtii* is spread over 40 sugar beet-growing countries throughout the world (McCarter et al. 2008).

Nematodes completely penetrate main and lateral roots in the elongation or root hair zones of a susceptible plant as motile infective second-stage juveniles (J2) which hatch in the soil from eggs contained within a protective cyst (cyst nematodes) or egg sac (root-knot nematodes). They penetrate the plant cell walls using their robust stylet. However, before the stylet penetrates, cell walls are degraded by a number of enzymes released from the nematode's subventral glands. These include β-1,4-endoglucanases (cellulases; Gao et al. 2001), a pectate lyase (Doyle and Lambert 2002) and an expansin (Qin et al. 2004). J2s migrate within the root cortex towards the vascular cylinder and induce remarkable changes in a number of host cells, to establish highly metabolically active feeding cells sustaining the nematode throughout its life cycle (syncytium for cyst nematodes; giant cell for root-knot nematodes; Davis et al. 2004, 2008; Fuller et al. 2008). After three additional molts, adult males emerge from the root and are attracted to the females, where fertilization occurs. At maturity, the female of a cyst nematode dies and the body is transformed into a light brown cyst where eggs and juveniles survive and remain dormant until root exudates stimulate juveniles to hatch and emerge from the cyst. By contrast, eggs of *Meloidogyne* spp. are released on the root surface in a protective gelatinous matrix.

Chemical control of nematodes is restricted. Most of the nematicides have been withdrawn from the market due to high environmental risks. Crop rotations with non-host plants including wheat, barley, corn, beans and alfalfa as well as nematode-resistant radish and mustard are functional, but often not economically practical. In this context, the breeding of resistant cultivars is the most promising alternative.

The majority of cloned nematode resistance genes originate from crop wild relatives. The first nematode R gene to be cloned was $Hs1^{pro\text{-}1}$ from sugar beet, which confers resistance against the sugar beet cyst nematode *H. schachtii* (Cai et al. 1997). Other cloned nematode R genes closely resemble known plant R genes in their domain structure. Four of these genes, *Mi-1*, *Hero*, *Gpa2* and *Gro1-4*, all cloned from tomato or potato relatives, fall into the NBS-LRR class of R genes (Williamson and Kumar 2006). The tomato genes *Mi-1* and *Hero*, respectively, confer broad-spectrum resistance to several root knot nematode species (Milligan et al. 1998; Vos et al. 1998) and to several pathotypes of the potato cyst nematodes *G. rostochiensis* and *G. pallida* (Ernst et al. 2002). *Mi* resistance was first transferred into commercial tomato cultivars in the 1950s (Gilbert et al. 1956). *Mi* also confers resistance to two totally unrelated parasites, the potato aphid *Macrosiphum euphorbiae* and the white fly *Bemisia tabaci* (Rossi et al. 1998; Nombela et al. 2003), whereas the potato genes *Gpa2* and *Gro1-4* mediate resistance to a narrow range of pathotypes of the potato cyst nematode *G. pallida* (van der Vossen et al. 2000;

Paal et al. 2004). So far, little is known about the action mode of the cloned nematode resistance genes. It is generally believed that these genes recognize nematode effectors triggering specific signaling pathways that lead to resistance responses. Agronomically more important nematode R genes are likely to be cloned in the near future, including the *H1* gene that confers resistance to *G. rostochiensis* in potato (Bakker et al. 2004) and the *Me* gene of pepper for resistance to *Meloidogyne* species (Djian-Caporalino et al. 2007).

10.2.3 Significance and Limitations of Plant Resistance Genes

Although the breeding of resistant cultivars is the most promising alternative for parasite control, there are several limitations for the use of natural nematode resistance genes in practice, generally.

1. Resistance is not complete. For example, *Hero A* is able to provide only partial resistance (>80%) to *G. pallida* (Ernst et al. 2002).
2. Resistance is conditionally expressed. The *Mi-1* mediated resistance is for example temperature-sensitive and breaks down above 28°C (Dropkin 1969).
3. Resistance genes are often effective against one or a limited range of species and introgression of such genes may confer yield penalties or undesirable agronomic traits (Panella and Lewellen 2007).
4. A major concern around resistance relying on a gene-for-gene relationship is when it is overcome by new virulent pathotypes even though the durability of R genes to sedentary plant nematodes has been generally high. The *H1* gene has been used in cultivated potato against *G. rostochiensis* for over 30 years in the UK but without the development of virulent population (Fuller et al. 2008).

Molecular identification and cloning of natural resistance genes make it feasible for a direct transfer of R genes into related susceptible cultivars or to other plants. Molecular markers can be developed, which can assist conventional breeding programs greatly, as demonstrated by the development of commercial soybean and potato cultivars resistant to *H. glycines* and *Globodera rostochiensis*, respectively (Starr et al. 2002). Broad resistance can be engineered by the pyramiding of different resistance genes in given species. In addition, a variety of defense-related genes from diverse sources is available for genetic engineering to enhance plant resistance to pests. These include genes specific for signaling components, defense-related genes with antimicrobial activity such as PR proteins, antifungal proteins (osmotin-, thaumatin-like), antimicrobial peptides (thionins, defensins, lectin, phytoalexins) as well as gene products that can enhance the structural defenses in the plant, such as peroxidase and lignin. The identification of global regulators of resistance response, 'master switches', offers the possibility to engineer broad disease resistance (Stuiver and Jerome 2001).

The techniques used to develop transgenic plants have improved dramatically in the past decade, allowing the development of new disease-resistant crops

(Dempsey et al. 1998) and transferring of the gene of interest across species that are difficult or impossible to cross. However, the transfer of resistance genes from a model to crop plants as well as between distantly related crops seems to be limited. Attempts to transfer *Mi*-mediated root-knot nematode resistance from tomato were unsuccessful (Williamson et al. 1998) and transfer of *Hero A* into a susceptible tomato cultivar conferred resistance to *Globodera* species but not in transgenic potato expressing the same construct (Sobczak et al. 2005). Exceptionally, expression of *Mi-1.2* in transgenic aubergine (*S. melongena*) resulted in significantly lower amounts of *Meloidogyne* reproduction and numbers of egg masses but had no anti-aphid effect (Goggin et al. 2006). It is generally believed that downstream components of the response cascade must be present to activate R gene-mediated resistance response in given species. Within species, significant variability in transgenic resistance may occur due to its genetic background and allelic status, the promoter used and the copy number of the transgen (Chen et al. 2006). The phenomenon termed 'restricted taxonomic functionality' (RTF) might reflect an inability of the R protein to interact with signal transduction components in the given host (Michelmore 2003).

10.3 Engineering of Insect and Nematode Resistance

Today, engineered insect and nematode resistance are becoming an essential part of a sustainable agriculture in both developing and developed countries worldwide. In 2007, insect-resistant plants based on the transgenic technology were grown on an area of 46 million hectares, more than half of it (26.9 million ha) with a stacked trait of herbicide- and insect-resistant seeds and 19.1 million hectares with insect resistance alone (James 2008). So far, several approaches are under discussion. The first one relies on expression of genes of interest in transgenic plants, whose products are non-phytotoxic but strong anti-parasitic, either lethal toxic or interfering with parasites after their take-up by parasites consequently affecting their development and reproduction. Such transgenes can encode enzymatic inhibitors that block physiological processes within the pest, toxic compounds that are then ingested, compounds that bind to signal molecules, enzymes that interfere with the nematode. Alternatively, the anti-feeding approach is aiming at breaking down the feeding structure by the introduction of genes encoding phytotoxic compounds like barnase or ribosome-inactivating proteins which disrupt feeding cells (Atkinson et al. 2003) or by the knockout of genes which are crucial for formation of the feeding structure or for nematode parasitism (Huang et al. 2006). Because this approach strictly relies on promoters as well as genes specific for nematode-feeding cells, the availability of these elements still remains the obstacle for its realization in practice (Atkinson et al. 2003). In the following, engineering insect and nematode resistance are discussed using anti-insect and anti-nematode genes.

10.3.1 Anti-Insect/Nematode Genes

10.3.1.1 Bt Toxins

Bt toxins have been known as molecules that are active against insects and nematodes since the beginning of the previous century. They are synthesized by the soil-borne gram-positive bacterium *Bacillus thuringiensis* (Bt). About 400 Bt toxins are known so far produced by diverse *B. thuringiensis* strains (Crickmore et al. 2009). All of them have a crystal structure, therefore named Cry toxins. Because of the natural origin of the toxins, they occupy the position of the world's leading bio-pesticide.

Cry proteins bind to glycoprotein receptors that are located within the membrane of target insects' epithelium and afterwards inserted irreversibly into the membrane leading to the formation of a pore. Reasonably, alterations of these glycoprotein receptors can cause as a reason for toxin resistance of insects to a particular Bt-protein (Knight et al. 1994, 1995; Malik et al. 2001; Griffitts et al. 2005). *B. thuringiensis* strains produce different crystal proteins with specific activity against distinct species: Cry1A, Cry1B, Cry1C, Cry1H, Cry2A against lepidoptera, Cry3A, Cry6A, Cry 12A, Cry13A against nematodes, Cry3A, Cry6A against coleoptera and Cry10A, Cry11A against diptera. The toxins are effective tools for controlling lepidopteran and coleopteran insect pests, but application of Bt toxins as an insecticide by spraying is not efficient because the protein is unstable and has no systemic effect. In contrast, when synthesized by transgenic plants, Cry protoxins are taken up by sucking insects. Within the insect gut, protoxins are proteolytically cleaved to produce the active toxin, finally leading to affection on epithelial cells. So far, Bt toxins have been introduced into a wide range of crop plants like soybean, maize and cotton (see Chaps. 16, 19, 25). More than 20 transgenic crop varieties carry Cry genes (Bruderer and Leitner 2003). For instance, Cry1Ab is integrated into the genome of the transgenic maize varieties MON810 and Bt176 (Bruderer and Leitner 2003), where it is particularly active against the european corn borer (*Ostrinia nubilalis*). In cotton the variety "Bollgard" expresses the Bt toxin Cry1Ac that is efficient for controlling the cotton bollworm (*Helicoverpa armigera*). To increase the expression levels of Bt toxins in transgenic plants, considerable changes to the Bt toxin genes are required such as change in codon-usage and the use of plant-specific processing signals in different events.

Even though immense advantages have been given by the use of Bt toxin in various transgenic crop plants (Romeis et al. 2006), the utilization of Bt toxin within transgenic plants is still controversially discussed, especially in Europe. Up to now, insect resistance against Bt toxins has not been observed under field conditions, only under laboratory conditions (Christou 2006), which is thought to be caused by a decreased fitness of resistant individuals (Christou et al. 2006; Soberón et al. 2007; Tabashnik et al. 2008). For instance, monitoring the pink bollworm (*Pectinophora gossypiella*) for eight years showed no increase of resistance to Bt (Tabashnik et al. 2005). The same result come from monitoring corn

borers (*Sesamia nonagrioides*, *Ostrinia nubilalis*) in Spain over a period of five years (Farinos et al. 2004). Furthermore, an overview about environmental effects of Bt proteins was made by Clark et al. (2005). A negative effect on non-target organisms under true conditions was not observed (Romeis et al. 2006). By contrast, a meta-analysis showed an increased abundance of non-target invertebrates on Bt-transgenic cotton and maize fields, compared to non-transgenic fields managed with insecticides, as reported by Marvier et al. (2007). It is generally believed that the durability of resistance will be extended, e.g. by establishing refuges with areas of susceptible plants or by growing transgenic crops with a multi-gene, multi-mechanistic resistance (Boulter et al. 1993). The strategy of pyramiding effector genes within crops has two follow two major aims. One potential effect is to broaden insecticidal activity by combining genes with different specificity to control insect and nematode pests. The second effect is to enhance the durability of genetically engineered plant resistance because single mutation events do not break the insecticidal effect (Maqbool et al. 2001). Developing different strategies to protect the insecticidal effect of Bt toxins remains a great challenge (McGaughey et al. 1992; Frutos et al. 1999; Bates et al. 2005).

The potential for Bt toxin as a nematicide was reported by Marroquin et al. (2000). A preliminary study with transgenic tomato plants expressing the Bt endotoxin CryIab after inoculation with *Meloidogyne* spp. resulted in a reduction in egg mass per gram of root of about 50% (Burrows and Waele 1997). The nematicidal effects were determined to result from a similar gut-damaging mechanism to that which occurs in insects: the activated toxin binds receptors in the intestine and forms a pore, causing lysis of the Gut (Wei et al. 2003; Li et al. 2007). Tomato hairy roots expressing the Bt crystal protein variant cry6A were challenged with *M. incognita* and supported significantly reduced amounts of nematode reproduction, although gall-forming ability was not affected (Li et al. 2007). The nematode feeding tube acts as a molecular sieve, permitting the uptake of certain molecules and excluding others. It is believed that root-knot nematodes are able to ingest larger molecules than cyst nematodes (Li et al. 2007). The size exclusion limit for *H. schachtii* has been determined to be approx. 23 kDa (Urwin et al. 1998). Therefore, the size exclusion limit (Böckenhoff and Grundler 1994; Urwin et al. 1997a, 1998; Li et al. 2007) severely restricts the agronomic application of transgenic Bt as a broad-spectrum nematode control strategy (Fuller et al. 2008).

10.3.1.2 Proteinase Inhibitors

The expression of proteinase inhibitors (PIs) of digestive proteinases in plants is a promising strategy of engineering insect and nematode resistance. Compared to Bt toxin, the beneficial properties of proteinase inhibitors are their small size and stability for their expression in transgenic plants. A direct proof of activity against insects was shown in transgenic tobacco plants which were resistant against a bud worm mediated by the expression of a trypsin inhibitor (Hilder et al. 1987).

PIs represent a well studied class of plant defense proteins which are generated within storage organs. Proteinase inhibitors are an important element of natural plant defense strategies (Ryan 1990) and are anti-feedants known to reduce the capacity of certain parasites to use dietary protein, so delaying their development and reducing their fecundity (Hilder et al. 1987). In addition, it has been shown that PIs are induced as part of defense cascades, e.g. by insect attack, mechanical wounding, pathogen attack and UV exposure (Ryan 1999). Different kinds of proteinase inhibitors are known to reduce the digestibility of the nutrients through oral uptake by insects and nematodes. The inhibitor binds to the active site of the enzyme to form a complex with a very low dissociation constant, thus effectively blocking the active site.

There are ten groups of PI characterized from plants spanning all four classes of proteinases: cysteine, serine, metallo- and aspartyl. The majority of proteinase inhibitors studied in the plant kingdom originates from three main families, namely Leguminosae, Solanaceae and Gramineae (Rao et al. 1991). The cowpea trypsin inhibitor (*CpTI*) is a serine inhibitor used in the first transgenic approach to confer insect resistance. CpTI in an amount of 1% of the solouble protein in the transgenic plant has an effect on the lepidopteran insect *Heliothis virens* in tobacco (Hilder et al. 1987) and inhibits insect development up to 50%. The gene was also transferred into potato, rice and other plants, where it showed similar activity. Another effective gene is the sweet potato trypsin inhibitor (*SpTI*) that is active against *Spodoptera litura* when it is expressed in tobacco and *Brassica* spp. (Yeh et al. 1997b; Ding et al. 1998). Another group of PI, cysteine proteinases, is common in animals, eukaryotic microorganisms and bacteria, as well as in plants. Recent studies have shown that other classes of proteases are also found in insect guts, such as cysteine proteinase (Wolfson and Murdock 1990). Brioschi et al. (2007) reported that adaptation of the insects to proteinase inhibitors appears through upregulation of proteinases, trypsins and chymotrypsins by insects.

The potential of plant proteinase inhibitors (PIs) for engineering nematode resistance has been demonstrated in several laboratories (Vain et al. 1998; Urwin et al. 2000; Cai et al. 2003). Both serine and cysteine proteinases are present in plant-parasitic nematodes (Koritsas and Atkinson 1994; Lilley et al. 1997). Their activities have been detected in the nematode intestine where they are involved in digestion of dietary proteins (Lilley et al. 1996). Broad nematode resistance has been achieved in potato plants by expressing a cystatin from rice, even when the proteinase inhibitor was preferentially expressed in feeding sites of *G. pallida* and *M. incognita* (Lilley et al. 2004). A cysteine proteinase inhibitor based transgenic resistance to the cyst nematode *Globodera pallida* in potato plants proved to be effective, even under field conditions (Urwin et al. 2001), demonstrating its great potential.

We demonstrated that sporamin, a tuberous storage protein of sweet potato is a functionally trypsin proteinase inhibitor. The full-length sporamin gene encodes a 23-kDa mature protein (Yao et al. 2001). It can be taken up through the feeding tube and the stylet and delivered within the nematode, where it can exhibit effective inhibition. After its transfer into the sugar beet hairy roots, a significant reduction of

developed females was observed in sporamin expressing roots but with variation in their inhibitory effects. Thereby the trypsin inhibitory activity was found to be a critical factor for nematode inhibition (Cai et al. 2003).

Nevertheless, there are no transgenic varieties carrying a proteinase inhibitor commercially available. It was discussed that parasites are able to modify there proteinase pattern and to bypass the inhibited protein digestion pathways (Broadway et al.1997; Giri et al. 1998). Thus, the source of the PI used in transgenic plants is critical to avoid development of insect insensibility (Ranjekar et al. 2003). Analogous to the case of Bt toxins, a combination of different PIs targeting a set of proteases would be a promising alternative to engineer a stable and broad resistance against insects and nematodes as well.

10.3.1.3 Lectins

Lectins are a structurally heterogeneous group of carbohydrate-binding proteins which play biological roles in many cellular processes. More than 500 different plant lectins have been isolated and (partially) characterized. Application of lectins as insecticidal protein has mainly been focused on homopteran, e.g. planthoppers, leafhoppers and aphids (Habibi J et al. 1993; Hussain et al. 2008). Because of their low level of susceptibility to proteinase inhibitors, lectins were considered to be a suitable insecticidal agent.

The toxic effect of lectins to insects and nematodes is still poorly understood. The proteins seem to bind to cells of the insect/nematode midgut disrupting the cell function like digestive processes and nutrient assimilation. Insect-feeding studies with purified lectins and experiments with transgenic plants confirmed that at least some lectins enhance the plant's resistance against insects and nematodes. Several lectins from plants have been reported to confer broad insect resistance against Lepidoptera, Coleoptera, Diptera and Homoptera (Carlini and Crossi-de-Sá 2002). A gene encoding a sugar-binding protein derived from pea (*Pisum sativum*) was the first example of a lectin which was used to generate transgenic plants with an enhanced insect resistance (Boulter et al. 1990). Another famous example of a lectin used in transgenic plants is the *Galanthus nivalis* agglutinin (GNA), which confers resistance against insects in rice, e.g. planthoppers (Rao et al. 1998; Nagadhara et al. 2004). Moreover, expression of GNA in potato has been shown to confer enhanced resistance to lepidopterans like *Lacanobia oleracea* and homopteran insects like aphids (Down et al. 1996; Gatehouse et al. 1997). Rapeseed was successfully transformed with a pea lectin, which leads to a reduced weight of pollen beetle larvae that was correlated to lectin expression (Melander et al. 2003).

Also, a significant reduction of *G. pallida* females was reported after transfer of the gene encoding the snowdrop (*Galanthus nivalis*) lectin GNA into potato plants (Burrows et al. 1997). It is believed that, analogous to insects, these proteins could be targeted to interact with the nematode at different sites: within the intestine; on the surface coat; or with amphidial secretions.

10.3.1.4 α-Amylase Inhibitors

α-Amylase inhibitors are inhibitory proteins that occur in the whole plant kingdom. These amylase inhibitors affect selectively α-amylase from insects, animals and microorganisms, but not amylases from plants. Groups of well characterized monomeric and dimeric α-amylase inhibitors were isolated from wheat, (*Triticum aestivum*) and common bean (*Phaseolus vulgaris*; Kashlan and Richardson 1981; Moreno and Chrispeels 1989; Octivio and Rigden 2002; Oneda et al. 2004). The function of these proteins within the plants/seeds has not yet been explained. They seem to be regulators of endogenous enzymes and part of the plant defense against insect attacks (Octivio and Rigden 2000). Analogous to the disruption of protein digestion by proteinase inhibitors, amylase inhibitors affect the carbohydrat metabolism of herbivorous insects. The potential of plant alpha-amylase inhibitors for engineering insect resistance was investigated in tobacco, pea and *Arabidopsis* (Carbonero et al. 1993; Schroeder et al. 1995). More promising results were obtained using the *Phaseolus* α-amylase inhibitor of BAAI in pea, maize (*Zea mays*) and coffee, leading to a decreased propagation of insect pests. Expressed in transgenic maize, BAAI showed an insecticidal activity to the western corn rootworm (*Diabrotica virgifera;* Titarenko and Chrispeels 2000). In pea (*Pisum sativum*), it was possible to reach a BAAI content of up to 3% of the soluble protein which mediates strong resistance to pea weevil (*Bruchus pisorum*; Schroeder et al. 1995; Morton et al. 2000). Pereira et al. (2006) reported an effect of BAAI in coffee on the coffee berry borer (*Hypothenemus hampei*), showing the broad potential of α-Amylase Inhibitors particularly against storage insect pests.

10.3.1.5 Chitinases and Others

A set of proteins from various organisms were tested for their activity against parasites. For instance, an insecticidal protein from scorpions enhances resistance to cotton bollworm (*Heliothis armigera*) larvae (Wu et al. 2008), toxins from endosymbionts of nematodes from the genus *Photorhabdus* and *Xenorhabdus* seems to have a broad insecticidal effect (Chattopadhyay et al. 2005).

Chitinases are known to be part of the plant defense system and are antifungal. A possible target is believed to be the nematode eggshell, which largely consists of chitin. We demonstrated recently that transgenic sugar beet roots and potato plants overexpressing a chitinase from the entomopathogenic fungus *Paecilomyces javanicus* confer broad-spectrum resistance to sedentary plant parasitic nematodes in transgenic sugar beet (*B. vulgaris*) and potato (*Solanum tuberosum*) plants (Thurau et al., unpublished data). The development of females was suppressed and the number of females was drastically reduced of both cyst nematodes *Heterodera schachtii* and *Globodera pallida*. In addition, the development of knots and egg sacks formed by root-knot nematode *Meloidogyne incognita* was also found to be severely affected. Although the mechanism underlying is not yet resolved and chitin has been reported to be present only in the egg shell of plant-parasitic

nematodes so far, our results strongly suggest an active role of chitin also in the parasitic process of various nematodes, thus providing an effective target for genetic engineering of broad nematode-resistant crops.

10.3.2 RNA Interference-Based Gene Silencing

The principle of RNA interference (RNAi) consists of a naturally based degradation of dsRNA as a part of protection against pathogen attack, particularly virus infection. This mechanism was discovered in the nematode *Caenorhabditis elegans*, leading to gene silencing through the occurrence of double-stranded RNA (dsRNA), mediating a downregulation of gene expression. Target RNA is degradated by enzyme complexes called DICER and RISC. The DICER endonucleases cuts double-stranded RNA into siRNAs of 21–23 nt. These small RNA molecules assemble at the RISC complex, which leads to the degradation of target RNA. Specificity of this mechanism depends on the sequence of the target-RNA molecule (for more details, see Chap. 5). For studying this mechanism in insects, *Drosophila melanogaster* functions as a model species (Wang et al. 2006).

An important aspect of RNAi in *C. elegans* is the ability to elicit phenotypic effects through the oral delivery of dsRNA molecules, either from solution or expressed within the bacteria upon which the nematode feeds, providing the new approach of engineering plant resistance to insect and nematode. Important advances have been made in the application of RNAi for nematode resistance over the past two years. Several reports demonstrated that plants expressing hairpin constructs targeting plant-parasitic nematode genes (Huang et al. 2006; Steeves et al. 2006; Yadav et al. 2006) display significant resistance to nematodes. Tobacco plant RNAi-induced silencing of *Meloidogyne* genes encoding a splicing factor and a component of a chromatin remodelling complex (Yadav et al. 2006) result in a high level of resistance to *M. incognita*. Huang et al. (2006) demonstrated the potential for engineering nematode resistance for plants by use of nematode parasitic genes. Transgenic *Arabidopsis* plants expressing the 16D10 sequence as a hairpin construct were found to be resistant to *Meloidogyne* species with a 63–90% reduction in the number of galls and as well as total egg production (Huang et al. 2006). The gene encodes a parasitism peptide which is probably involving the early signaling events in the formation of giant cells. Because of a high degree of homology between the 16D10 sequences of different *Meloidogyne* species, broad-range resistance against *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla* is induced. Although there are reports of the technology being used to silence genes of cyst nematodes (Steeves et al 2006; Valentine et al. 2007), less success has been reported by many workers attempting to engineer resistance to these species (Gheysen and Vanholme 2007). One explanation for these results could be differences in the maximum size of molecule that each species is able to ingest from the plant cell owing to the size exclusion limits imposed by the feeding tube, as

discussed above. It is reasonable to believe that the cyst nematode feeding tube may not allow an efficient uptake of the construct carrying the target molecule.

Also, RNAi proved to be be a suitable method to control coleopteran insect pests, as shown by Mao et al. (2007) silencing a cytochrome 450 monooxygenase gene (CYP6AE14) in the cotton bollworm (*Helicoverpa armigera*) and impairing tolerance of bollworm larval to the cotton metabolite gossypol. Baum et al. (2007) shown similar results in the pathosystem western corn rootworm *Diabraotica vigifera*, where post-transcriptional gene silencing of several genes was induced and larval mortality was investigated in a feeding assay with transgenic corn plants and roots as well. Potential progress in the field of insect resistance, mediated by the host plant's delivery siRNA molecules, is restricted by the fact that insects lack genes encoding RNA-dependant RNA polymerase (RdRP), an enzyme necessary for the systemic activity of RNAi-mediated gene silencing (transitive RNAi; Gordon and Waterhouse 2007; Price and Gatehouse 2008). Nevertheless the possibilities of the RNAi mechanism for engineering insect resistance still have to be determined in the future.

10.4 Conclusions

During the past years, proteins like Bt toxins, proteinase inhibitors, lectins and amylase inhibitors were intensively investigated in respect of their anti-insect and nematode efficiency both in laboratory and in field trials. Significant control effects to parasitic pests have been achieved and demonstrated with different transgenic crop species. Crop species expressing the *Cry* proteins from *Bacillus thuringiensis* are worldwide commercialized with an enormous success. So far there is no commercialized transgenic crop for nematode resistance available. Control of insect and nematode pests, particular in developing countries, is still a great challenge for agriculture. Obvious advantages of engineered resistance like independence of genotype, reducing pesticide/nematicide application and improving human health as well as protecting the environment meet increasing demands of modern agricultural practices, especially with a global climate change for concern. Nevertheless, new genetic variability, molecular knowledge of the resistance mechanisms and new target proteins as well as novel engineering technologies is needed. In this context, fundamental research on molecular plant–parasite interaction will provide new approaches.

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