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Perspective

Transgenic plants for insect pest control: a forward looking scientific perspective

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Abstract

One of the first successes of plant biotechnology has been the creation and commercialisation of transgenic crops exhibiting resistance to major insect pests. First generation products encompassed plants with single insecticidal Bt genes with resistance against major pests of corn and cotton. Modelling studies predicted that usefulness of these resistant plants would be short-lived, as a result of the ability of insects to develop resistance against single insecticidal gene products. However, despite such dire predictions no such collapse has taken place and the acreage of transgenic insect resistance crops has been increasing at a steady rate over the 9 years since the deployment of the first transgenic insect resistant plant. However, in order to assure durability and sustainability of resistance, novel strategies have been contemplated and are being developed. This perspective addresses a number of potentially useful strategies to assure the longevity of second and third generation insect resistant plants.

Introduction

Crop losses due to insect pests, estimated at 10–20% for major crops, are a significant factor in limiting food production. Engineering crop plants for enhanced resistance to insect pests has been one of the real successes of transgenic technology. Currently >22 million hectares are planted globally to crops expressing *Bacillus thuringiensis* (Bt) δ-endotoxins (James, 2004). To date no insect pests have developed field resistance to transgenic Bt crops despite dire predictions to the contrary. This may be due in part, to successful insect resistance management strategies (Bates et al., 2005). As adoption rates of insect resistant crops rise globally, selection pressure for the development of insect pests resistant to transgenic insecticidal gene products will increase.

It is increasingly clear that complementary durable pest control strategies are necessary, to assure we do not compromise the benefit(s) provided by transgenic-based insect pest control strategies. The next generation of insect-resistant transgenic crops has the potential to achieve this goal. However, these should not be viewed as a panacea to insect resistance problems but as an opportunity to integrate management strategies (Integrated Pest Management, IPM and Integrated Resistance Management, IRM) with the new technologies and thus achieve more sustainable agricultural systems.

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Alternative strategies

Expressing insecticidal proteins from sources other than *Bacillus thuringiensis* in crop plants either alone or in conjunction with Bt genes should reduce the likelihood for development of resistance to these toxins within insect pest populations. Thus toxins from other insect pathogens are also opening up new routes to transgenic pest control. *Photorhabdus* and *Xenorhabdus* spp. are bacterial symbionts of entomopathogenic nematodes and in contrast to endotoxins from Bt that are specific to certain group of insects, *Photorhabdus* toxins are lethal to a wide range of insects (Chattopadhyay et al., 2005) and thus may confer a broader spectrum of insect resistance than that provided by Bt alone. Other strategies currently being developed include those based on the expression of insecticidal proteins produced by foreign genes from plants (enzyme inhibitors, lectins) (Tinjuangjun et al., 2000; Loc et al., 2002; Rahbe et al., 2003; Abdeen. et al., 2005) and animals, including insects (e.g., biotin-binding proteins (Burgess et al., 2002), neurohormones (Fitches et al., 2002), enzyme inhibitors (Christeller et al., 2002). However, total pest control at the same level as is routinely provided by Bt toxins, is rarely achieved and phytophagous pests have been shown to be able to adapt to plant proteins, especially plant proteinase inhibitors (De Leo et al., 2001).

Such strategies are dependent on the identification of novel genes encoding insecticidal products with suitable characteristics for use in transgenic crops. Interesting recent developments include the use of novel proteins from insect biological control agents and insect hormones to generate transgenic crops. A teratocyte secretory protein from a hymenopteran endoparasitoid (a parasitic wasp often used in biocontrol programs) has been expressed in transgenic tobacco and shown to increase resistance to lepidopteran pests (Maiti et al., 2003). Similar protection has also been achieved with insect peptide hormones (Tortiglione et al., 2003). Interestingly they replaced the systemin peptide region of prosystemin with the insect peptide and showed that this resulted in the delivery of short biologically active peptides.

Reliance on the expression of a single gene product for pest control is a relatively short-term strategy that parallels the use of exogenously applied chemical pesticides. Thus pyramiding (stacking) of genes encoding different Bt toxins has been developed as a method for preventing development of pest resistance, and for conferring greater levels of pest control (Boulter et al., 1990; Maqbool et al., 2001; Zhao et al., 2003). For example, corn lines have recently been developed (Moellenbeck et al., 2001; Ostlie, 2001) co-expressing two δ-endotoxins from Bt for resistance to corn rootworm. Hybrid proteins have also been developed to enhance and extend the activity of Bt toxins. The use of a single Bt toxin in a crop is limited in that many insects attack a single crop and toxins generally show very high specificity towards a single pest species. Therefore toxins have been engineered to modify their receptor recognition and pore formation. Each toxin consists of three domains. Domain I is involved in membrane insertion and pore formation. Domains II and III are both involved in receptor recognition and binding. Additionally, a role for domain III in pore function has been found. This approach has proved successful in both enhancing activity (Karlova et al., 2005) and extending host range (Singh et al., 2004).

Such hybrid/fusion proteins offer an alternative/complementary strategy to address potential limitations in conventional transgenic insect pest control.

Fusion proteins

Recognition of binding sites in the insect gut is an important factor determining the toxicity of Bt. Enhancing toxin binding capabilities should extend host range and delay resistance. In our most recent work we devised a strategy where Bt was covalently fused to the non-toxic B-chain of ricin (RB) Mehlo et al. (2005). Bt is believed to bind primarily to aminopeptidase N or cadherin membrane proteins. The generation of a fusion protein with RB extends binding to include specific glycoproteins. Bioassays with transgenic plants expressing the BtRB show that the addition of the RB binding domain provided a wider repertoire of receptor sites within target species and significantly enhances the toxicity of Bt. For example survival of the stem borer *Chilo suppressalis* feeding on transgenic maize expressing the fusion was reduced by greater than 50% in comparison to Bt Cry1Ac alone. Expression of the fusion in rice showed similar levels of protection with both transgenic lines dramatically reducing insect growth, with corresponding reductions in the level of tissue damage. Bioassays against Bt tolerant *Spodoptera littoralis* confirmed the insensitivity of the insect to the Bt toxin when expressed individually but show that fusion of the toxin to the ricin domain extended the range of toxicity of Cry1Ac to include this species. Furthermore, we showed that Bt toxins with additional binding domains can generate unique insecticidal activity giving protection against attack from the Homopteran leafhopper *Cicadulina mbila*.

This strategy has shown great potential beyond just extending the toxicity of Bt. Zhu-Salzman et al. (2003) have generated fusion proteins with anchor regions to tether insecticidal proteins to the insect gut epithelium. Using the legume lectin rGSII they proposed a system to combat the ability of certain insect species to activate protease inhibitor insensitive proteolytic enzymes. The soybean cysteine protease inhibitor soyacystatin N (scN) was covalently linked to the GlcNAc specific legume lectin using a naturally occurring linker region from the potato multicystatin. In this instance the fusion protein not only has a novel binding ability that is proposed to initiate a concentration effect by localising the inhibitor at the anterior of the gut, but the fused lectin moiety additionally offers a degree of protection to the insecticidal moiety by blocking the access of scN-insensitive proteases, therefore preventing proteolytic destruction of the cystatin.

Targeting of fusion proteins using snowdrop lectin

The targeting of toxic proteins becomes more of a challenge to developing pest control strategies when the target receptor is separated from the primary delivery site by cellular membranes. Pest management strategies based on neuropeptides potentially offer a high degree of biological activity, target specificity and environmental compatibility that are lacking in conventional neurotoxic insecticides. However, insect neuropeptides are unlikely to be rapidly absorbed through the insect cuticle, and are prone to proteolysis and rapid degradation both in the environment and within the insect gut, thus limiting their usefulness as topically applied insecticidal sprays. The discovery that following ingestion the snowdrop lectin (GNA) remains stable and active within the insect gut and is able to cross the gut epithelium offers the possibility for its use as a ‘carrier molecule’ to deliver other peptides to the circulatory system of target insect species. This strategy has been employed by Fitches et al. (2002) to deliver the insect neuropeptide hormone allatostatin to the haemolymph of the lepidopteran *Lacanobia oleracea*. The protective role of this strategy was demonstrated by bioassays of *L. oleracea* with the fusion protein in potato leaf based artificial diet, where the results show a consistent and significant reduction in mean larval weight when compared to the controls. Using this delivery system Fitches et al. (2004) also demonstrate that GNA can be used to deliver an insecticidal peptide isolated from the venom of the spider *Segestria florentina* (SFI1) to the haemolymph of *L. oleracea*. In feeding studies neither the GNA nor the SFI1 moieties alone showed acute toxicity, however feeding SFI1/GNA fusion at 2.5% of dietary proteins was insecticidal to first stage larvae, causing 100% mortality after 6 days. This spider venom neurotoxin is believed to irreversibly block the pre-synaptic neuromuscular junctures. Such venom toxins show high degrees of specificity and thus lend themselves to environmentally benign pest management strategies.

Manipulation of endogenous defense

Ecologists have long understood that plants exhibit multi-mechanistic resistance towards herbivores but the molecular mechanisms underpinning these complicated responses have remained elusive (Baldwin et al., 2001). However, recent studies investigating the plant’s herbivore-induced transcriptome, using microarrays and differential display technologies, have provided novel insights into plant–insect interactions. The jasmonic acid cascade plays a central role in transcript accumulation in plants exposed to herbivory (Hermsmeier et al., 2002; Bailey et al., 2005). A single microarray based study revealed that the model plant *Arabidopsis* undergoes changes in levels of over 700 mRNAs during the defence response (Schenk et al., 2000). In contrast only 100 mRNAs were up-regulated by spider mite (*Tetranicus urticae*) infestation in lima bean (*Phaseolus lunatus*), although a further 200 mRNAs were up-regulated in an indirect response mediated by feeding-induced volatile signal molecules (Arimura et al., 2000). The insect herbivore has an additional effect on the plant's response over that caused by mechanical tissue damage (Stout et al., 1999). Analysis of timing, dynamics, and regulation of the expression of 150 genes in leaves of *Arabidopsis* showed that many genes strongly induced by mechanical damage were induced less, or not at all, when the plant was attacked by the lepidopteran pest *Pieris rapae*.

Whereas most phytophagous insects cause extensive damage to plant tissues when feeding, many insects of the order Homoptera feed from the contents of vascular tissues by inserting a stylet between overlying cells, thus limiting cell damage and minimising induction of a wound response. Moran and Thompson (2001) demonstrated that phloem-feeding by the aphid *Myzus persicae* induced expression of genes associated with salicylic acid responses to pathogens as well as a gene involved in the jasmonic acid mediated response pathway. More recently Zhang et al. (2004) demonstrate that upon attack by the piercing–sucking insect, brown planthopper, BPH (*Nilaparvata lugens*), rice genes that were strongly regulated were grouped in the categories of signalling pathways, oxidative stress/apoptosis, wound-response, drought-inducible and pathogen-related proteins. Those related to the flavenoid pathway, aromatic amino acid metabolism and the octadecanoid acid pathway were either unchanged or down-regulated. Thus BPH induces plant responses which would take part in a jasmonic acid independent pathway and crosstalk with those related to abiotic stress, pathogen invasion and phytohormone signalling pathways. Recently Divol et al. (2005) demonstrated that 126 different genes were up-regulated in response to aphid feeding. The model system used was *Myzus persicae* feeding on celery (a plant from which phloem is easily separated from the surrounding tissues); they found that the phloem is actively involved in multiple adjustments away from the feeding site and that specific mechanisms are displayed that are not induced in other tissues. The genes affected belonged to various functional categories and were induced systemically in the phloem following aphid infestation. Cell wall modification, water transport, vitamin biosynthesis, photosynthesis, carbon assimilation and nitrogen and carbon mobilisation were all up-regulated. Furthermore, analysis of phloem and xylem responses indicated that a component of the response was more developed in the phloem, however, it was different from responses driven by pathogen infection.

Signalling

The studies of Baldwin and his group on the interaction between insect herbivores and tobacco (*Nicotiana attenuata*) have provided new insights into the molecular bases of plant defence. They estimate that approximately 500 mRNAs constitute the insect-responsive transcriptome in tobacco (Hermsmeier et al., 2001). However, many of these genes are of unknown function, and many changes in gene expression do not represent induction of defence-related proteins. Photosynthetic genes, for example, are down-regulated in tobacco plants in response to insect attack. Further microarray analysis (Hui et al., 2003) has demonstrated putative up-regulation of defence-associated and down-regulation of growth-associated transcripts. This analysis provided evidence for the simultaneous activation of salicylic acid, ethylene, cytokinin and jasmonic acid-regulated pathways during herbivore attack. Similar co-activation of numerous signalling cascades in response to various stresses has been found in *Arabidopsis* (Chen et al., 2002) and supports the idea of a network of interacting signal cascades. Microarray analysis also identified direct defensive responses in dramatic increases in PI transcripts, and increases in transcripts encoding putrescine *N*-methyl transferase (which catalyses the first committed step in the biosynthesis of nicotine), as well as metabolic commitment to terpenoid based indirect defences.

Deciphering of the signals regulating herbivore-responsive gene expression will afford many opportunities to manipulate the response. Signalling molecules such as salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) do not activate defences independently by linear cascades, rather establish complex interactions that determine specific responses. Knowledge of these interactions can be exploited in the rational design of transgenic plants with increased disease/insect resistance (Rojo et al., 2003).

It is, however, only recently that Mewis et al., (2005) linked these interacting signalling systems directly with insect attack. The glucosinolate profile of mutant and transgenic *Arabidopsis* compromised in the three major signalling pathways was characterised in response to chewing and phloem feeding pests. Glucosinolate (GS) concentrations were reduced in blocked JA and enhanced SA mutants. Blocking SA increased them. No impact on glucosinolate concentration was found in ET blocked signalling. Glucosinolate accumulation was shown to increase in response to insect feeding and insect performance was negatively related to GS levels. These major pathways modulate *Arabidopsis* GS accumulation in response to both phloem feeding and chewing insects, often antagonistically. However, engineering natural pathways for plant improvement is limited by a lack of understanding of their biochemistry, and by the need for co-ordinate regulation of multiple gene activities (Dixon, 2005).

Regulation

The manipulation of plant traits in agricultural biotechnology would be greatly facilitated if endogenous genes of interest could be turned on or off in a controlled or selective manner (Segal et al., 2003). Manipulation of gene regulation may be achieved utilising natural and artificial transcription factors. In nature the expression of eukaryotic genes is tightly regulated at both the transcriptional and translational level, much of this control is achieved through DNA-binding transcription factors. Due to whole genome sequencing projects numerous putative plant transcription factors have been identified. Although characterisation is still at a very early stage, this strategy has significant potential. A transgenic line of *Arabidopsis* expressing a MYB transcription factor of phenylpropanoid biosynthesis significantly increased resistance to lepidopteran leaf feeding (Johnson & Dowd, 2004). While these results indicate that a single gene regulator can activate a defensive pathway sufficient to produce increased disease resistance, a significant cost in plant productivity was observed. Similarly zinc-finger-based artificial transcription factors (the zinc finger domain is the most common DNA-binding motif in nature) are providing a promising new method for manipulation of gene regulation (Segal et al., 2003). While it is not yet possible to use them broadly as gene-specific tools to regulate endogenous gene expression some regulatory factors are already being explored for agricultural biotechnology applications.

Conclusions

Pest control in modern agriculture has undergone a true revolution, and is increasingly moving away from reliance on exogenous pesticides towards more sustainable and environmentally benign practices. Whilst exploitation of plant endogenous defense systems and resistant germplasm is an intergral component of IPM-based strategies, this is not without its drawbacks. Pest control is often partial and resistance is only effective against specific pest biotypes. However, the introduction of foreign genes conferring insect resistance to crop plants has been a major success in terms of levels of protection afforded by expression of Bt toxins. It is imperative to assure that transgenic insect pest control is not compromised by the development of resistance in pest populations. The next generation of insect-resistant transgenic plants must be designed to delay/prevent the onset of resistance and thus provide more durable levels of crop protection. The goal of achieving multi-mechanistic resistance in crops is increasingly achievable with agricultural biotechnology. Such a goal may be realised using hybrid/fusion proteins, which extend the efficacy of single toxins, or via exploitation of induced responses, However, if endogenous defense systems are to be fully exploited, a much greater level of knowledge of the regulation of complex pathways is required.

Field evaluation of transgenic plants containing insecticidal genes, as exemplifed by Eizaguirre et al. (2006, this issue of Transgenic Research), is vital as a component of the overall process of creating and deploying insect resistant transgenic plants that are useful and sustainable.

References

Abdeen A, Virgos A, Olivella E, Villanueva J, Aviles X, Gabarra R and Prat S (2005) Multiple insect resistence in transgenic tomato plants over-expressing two families of plant proteinase inhibitors. *Plant Mol Biol* **57**: 189–202.

Arimura G, Tashiro K, Kuhara S, Nishioka T, Ozawa R and Takabayashi J (2000) Gene responses in bean leaves induced by herbivory and by herbivore-induced volatiles. *Biochem Biophys Res Commun* **277**: 305–310.

Bailey BA, Strem MD, Bae H, De Antunez Mayolo G and Guiltinan MJ (2005) Gene Expression in leaves of *Theobroma cacao* in response to mechanical wounding, ethylene and/or methyl jasmonate. *Plant Sci* **168**: 1247–1258.

Baldwin IT, Halitschke R, Kessler A and Schittko U (2001) Merging molecular and ecological approaches in plant–insect interactions. *Curr Opin Plant Biol* **4**: 351–358.

Bates SL, Zhao JZ, Roush RT and Shelton AM (2005) Insect resistance management in GM crops: past, present and future. *Nat Biotechnol* **23**(1): 57–62.

Boulter D, Edwards GA, Gatehouse AMR, Gatehouse JA and Hilder VA (1990) Additive protective effects of different plant-derived insect resistance genes in transgenic tobacco plants. *Crop Protect* **9**: 351–354.

Burgess EPJ, Malone LA, Christeller JT, Lester MT, Murray C, Philip BA, Phung MM and Tregidga EL (2002) Avidin expressed in transgenic tobacco leaves confers resistance to two noctuid pests, *Helicoverpa armigera* and *Spodoptera litura*. *Transgenic Res* **11**: 185–198.

Chattopadhyay A, Bhatnagar NB and Bhatnagar R (2005) Bacterial insecticidal toxins. *Crit Rev Microbiol* **30**(1): 33–54.

Chen WQ, Provart NJ, Glazebrook J, Katagiri F, Chang HS, Eulgem T, Mauch F, Luan S, Zou GZ and Whitham SA (2002) Expression profile matrix of *Arabidopsis* transcription factor genes suggests their putative functions in response to environmental stresses. *Plant Cell* **14**: 559–574.

Christeller JT, Burgess EPJ, Mett V, Gatehouse HS, Markwick NP, Murray C, Malone LA, Wright MA, Philip BA and Watt D (2002) The expression of a mammalian proteinase inhibitor, bovine spleen trypsin inhibitor in tobacco and its effects on *Helicoverpa armigera* larvae. *Transgenic Res* **11**: 161–173.

De Leo F, Bonade-Bottino M, Ceci LR, Gallerani R and Jouanin L (2001) Effects of a mustard trypsin inhibitor expressed in different plants on three Lepidopteran pests. *Insect Biochem Mol Biol* **31**(6–7): 593–602.

Divol F, Vilaine F, Thibivilliers S, Amselem J, Palauqui JC, Kusiak C and Dinant S (2005) Systemic response to aphid infestation by *Myzus persicae* in the phloem of *Apium graveolens*. *Plant Mol Biol* **57**(4): 517–540.

Dixon RA (2005) Engineering of plant natural product pathways. *Curr Opin Plant Biol* **8**(3): 329–336.

Eizaguirre M, Albajes R, Lopez C, Jordi E, Lumbierres B and Pons X (2006) Six years after the commercial introduction of Bt maize in Spain: field evaluation, impact and future prospects. *Transgenic Res* **15**:1–12.

Fitches E, Audsley N, Gatehouse JA and Edwards JP (2002) Fusion proteins containing neuropeptides as novel insect contol agents: snowdrop lectin delivers fused allatostatin to insect haemolymph following oral ingestion. *Insect Biochem Mol Biol* **32**: 1653–1661.

Fitches E, Edwards MG, Mee C, Grishin E, Gatehouse AMR, Edwards JP and Gatehouse JA (2004) Fusion proteins containing insect-specific toxins as pest control agents: snowdrop lectin delivers fused insecticidal spider venom toxin to insect haemolymph following oral ingestion. *J Insect Physiol* **50**(1): 61–71.

Guo HN, Chen XY, Zhang HL, Fang RX, Yuan ZQ, Zhang ZS and Tian YC (2004) Characterization and activity enhancement of the phloem-specific pumpkin PP2 gene promoter. *Transgenic Res* **13**(6): 559–566.

Hammond-Kosack KE and Parker JE (2003) Deciphering plant–pathogen communication: fresh perspectives for molecular resistance breeding. *Curr Opin Biotechnol* **14**: 177–193.

Heise A, Lippok B, Kirsch C and Hahlbrock K (2002) Two immediate-early pathogen-responsive members of the *AtCMPG* gene family in *Arabidopsis thaliana* and the W-box-containing elicitor-response element of *AtCMPG1*. *Proc Natl Acad Sci USA* **99**: 9049–9054.

Hermsmeier D, Schittko U and Baldwin IT (2001) Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. I. Large-scale changes in the accumulation of growth- and defence-related plant mRNAs. *Plant Physiol* **125**: 683–700.

Hooker TS, Millar AA and Kunst L (2002) Significance of the expression of the CER6 condensing enzyme for cuticular wax production in *Arabidopsis*. *Plant Physiol* **129**: 1568–1580.

Hui DQ, Iqbal J, Lehmann K, Gase K, Saluz HP and Baldwin IT (2003) Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*: V. Microarray analysis and further characterization of large-scale changes in herbivore-induced mRNAs. *Plant Physiol* **131**: 1877–1893.

James C (2004) Global Status of Commercialised Transgenic Crops ISAAA Briefs No.32: Preview. ISAAA, Ithaca, NY.

Johnson ET and Dowd PF (2004) Differentially enhanced insect resistance, at a cost, in *Arabidopsis thaliana* constitutively expressing a transcription factor of defensive metabolites. *J Agric Food Chem* **52**(16): 5135–5138.

Karlova R, Weemen-Hendriks M, Naimov S, Ceron J, Dukiandjiev S and de Maagd RA (2005) *Bacillus thuringiensis* delta-endotoxin Cry1Ac domain III enhances activity against *Heliothis virescens* in some, but not all Cry1-Cry1Ac hybrids. *J Invertebr Pathol* **88**(2): 169–172.

Kirsch C (2001) A highly specific pathogen-responsive promoter element from the immediate-early activated *CMPG1* gene in *Petroselinum crispum*. *Plant J* **26**: 217–227.

Maiti IB, Dey N, Pattanaik S, Dahlman DL, Rana RL and Webb BA (2003) Antibiosis-type insect resistance in transgenic plants expressing a teratocyte secretory protein (TSP14) gene from a hymenopteran endoparasite (*Microplitis croceipes*). *Plant Biotechnol J* **1**(3): 209–219.

Maqbool SB, Riazuddin S, Loc NT, Gatehouse AMR, Gatehouse JA and Christou P (2001) Expression of multiple insecticidal genes confers broad resistance against a range of different rice pests. *Mol Breed* **7**: 85–93.

Mehlo L, Gahakwa D, Nghia PT, Loc NT, Capell T, Gatehouse JA, Gatehouse AMR and Christou P (2005) An alternative strategy for sustainable pest resistance in genetically enhanced crops. *Proc Natl Acad Sci USA* **102**(22): 7812–7816.

Mewis I, Appel HM, Hom A, Raina R and Schultz JC (2005) Major signaling pathways modulate *Arabidopsis* glucosinolate accumulation and response to both phloem-feeding and chewing insects. *Plant Physiol* **138**(2): 1149–1162.

Michelmore RW (2003) The impact zone: genomics and breeding for durable disease resistance. *Curr Opin Plant Biol* **6**: 397–404.

Moellenbeck DJ, Peters ML, Bing JW, Rouse JR, Higgins LS, Sims L, Nevshemal T, Marshall L, Ellis RT and Bystrak PG (2001) Insecticidal proteins from *Bacillus thuringiensis* protect from corn rootworms. *Nat Biotechnol* **19**: 668–672.

Moran PJ and Thompson GA (2001) Molecular responses to aphid feeding in *Arabidopsis* in relation to plant defense pathways. *Plant Physiol* **125**(2): 1074–1085.

Nagadhara D, Ramesh S, Pasalu IC, Rao YK, Sarma NP, Reddy VD and Rao KV (2004) Transgenic rice plants expressing the snowdrop lectin gene (gna) exhibit high-level resistance to the whitebacked planthopper (*Sogatella furcifera*). *Theor Appl Genet* **109**: 1399–1405.

Okumoto S, Koch W, Tegeder M, Fischer WN, Biehl A, Leister D, Stierhof YD and Frommer WB (2004) Root phloem-specific expression of the plasma membrane amino acid proton co-transporter AAP3. *J Exp Bot* **55**: 2155–2168.

Ostlie K (2001) Crafting crop resistance to corn rootworms. *Nat Biotechnol* **19**: 624–625.

Padidam M (2003) Chemically regulated gene expression in plants. *Curr Opin Plant Biol* **6**: 169–177.

Retnakaran A, Krell P, Feng QL and Arif B (2003) Ecdysone agonists: Mechanism and importance in controlling insect pests of agriculture and forestry. *Arch Insect Biochem Physiol* **54**: 187–199.

Rao KV, Rathore KS, Hodges TK, Fu X, Stoger E, Sudhakar D, Williams S, Christou P, Bharathi M, Bown DP, Powell KS, Spence J, Gatehouse AMR and Gatehouse JA (1998) Expression of snowdrop lectin (GNA) in transgenic rice plants confers resistance to rice brown planthopper. *Plant J* **15**(4): 469–477.

Rojo E, Solano R and Sanchez-Serrano JJ (2003) Interactions between signaling compounds involved in plant defence. *J Plant Growth Regul* **22**: 82–98.

Rushton PJ, Reinstadler A, Lipka V, Lippok B and Somssich IE (2002) Synthetic plant promoters containing defined regulatory elements provide novel insights into pathogen- and wound-induced signalling. *Plant Cell* **14**(4): 749–762.

Schenk PM, Kazan K, Wilson I, Anderson JP, Richmond T, Somerville SC and Manners JM (2000) Coordinated plant defence responses in *Arabidopsis* revealed by microarray analysis. *Proc Natl Acad Sci USA* **97**: 11655–11660.

Segal DJ, Stege JT and Barbas CF (2003) Zinc fingers and a green thumb: manipulating gene expression in plants. *Curr Opin Plant Biol* **6**(2): 163–168.

Singh PK, Kumar M, Chaturvedi CP, Yadav D and Tuli R (2004) Development of a hybrid delta-endotoxin and its expression in tobacco and cotton for control of a polyphagous pest *Spodoptera litura*. *Transgenic Res* **13**(5): 397–410.

Stout MJ, Fidantsef AL, Duffey SS and Bostock RM (1999) Signal interactions in pathogen and insect attack: systemic plant-mediated interactions between pathogens and herbivores of the tomato, *Lycopersicon esculentum*. *Physiol Mol Plant Pathol* **54**: 115–130.

Tomsett B, Tregova A, Garoosi A and Caddick M (2004) Ethanol-inducible gene expression: first step towards a new green revolution? *Trends Plant Sci* **9**: 159–161.

Tortiglione C, Fogliano V, Ferracane R, Fanti P, Pennacchio F, Monti LM and Rao R (2003) An insect peptide engineered into the tomato prosystemin gene is released in transgenic tobacco plants and exerts biological activity. *Plant Mol Biol* **53**(6): 891–902.

Zhang F, Zhu L and He GC (2004) Differential gene expression in response to brown planthopper feeding in rice. *J Plant Physiol* **161**(1): 53–62.

Zhao J-Z, Cao J, Li Y, Collins HL, Roush R, Earle ED and Shelton AM (2003) Transgenic plants expressing two *Bacillus thuringiensis* toxins delay insect resistance evolution. *Nat Biotechnol* **21**: 1493–1497.

Zhu-Salzman K, Ahn JE, Salzman RA, Koiwa H, Shade RE and Balfe S (2003) Fusion of a soybean cysteine protease inhibitor and a legume lectin enhances anti-insect activity synergistically. *Agric Forest Entomol* **5**(4): 317–323.