Zeitschriftenaufsatz

Abdellatef, Eltayb; Kamal, Nasrein Mohamed; Tsujimoto, Hisashi (2021):

Tuning Beforehand: A Foresight on RNA Interference (RNAi) and In Vitro-Derived dsRNAs to Enhance Crop Resilience to Biotic and Abiotic Stresses.

In: *International journal of molecular sciences* 22 (14). DOI: 10.3390/ijms22147687.

Abstract:

Crop yield is severely affected by biotic and abiotic stresses. Plants adapt to these stresses mainly through gene expression reprogramming at the transcriptional and post-transcriptional levels. Recently, the exogenous application of double-stranded RNAs (dsRNAs) and RNA interference (RNAi) technology has emerged as a sustainable and publicly acceptable alternative to genetic transformation, hence, small RNAs (micro-RNAs and small interfering RNAs) have an important role in combating biotic and abiotic stresses in plants. RNAi limits the transcript level by either suppressing transcription (transcriptional gene silencing) or activating sequence-specific RNA degradation (post-transcriptional gene silencing). Using RNAi tools and their respective targets in abiotic stress responses in many crops is well documented. Many miRNAs families are reported in plant tolerance response or adaptation to drought, salinity, and temperature stresses. In biotic stress, the spray-induced gene silencing (SIGS) provides an intelligent method of using dsRNA as a trigger to silence target genes in pests and pathogens without producing side effects such as those caused by chemical pesticides. In this review, we focus on the potential of SIGS as the most recent application of RNAi in agriculture and point out the trends, challenges, and risks of production technologies. Additionally, we provide insights into the potential applications of exogenous RNAi against biotic stresses. We also review the current status of RNAi/miRNA tools and their respective targets on abiotic stress and the most common responsive miRNA families triggered by stress conditions in different crop species.

Schlagwörter:

Animals; Crop Production/methods; Crop Protection/methods; Crops, Agricultural/genetics; gene silencing; insect control; Insecta/genetics/pathogenicity; MicroRNAs/genetics; Plant Defense Against Herbivory/genetics; RNA Interference; RNA, Double-Stranded/genetics; RNA, Plant/genetics; RNA, Small Interfering/genetics; Stress, Physiological/genetics

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.2.2 spray (SIGS); 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Ahmed, Firoz; Senthil-Kumar, Muthappa; Dai, Xinbin; Ramu, Vemanna S.; Lee, Seonghee; Mysore, Kirankumar S.; Zhao, Patrick Xuechun (2020):

pssRNAit: A Web Server for Designing Effective and Specific Plant siRNAs with Genome-Wide Off-Target Assessment.

In: *Plant physiology* 184 (1), S. 65–81. DOI: 10.1104/pp.20.00293.

Abstract:

We report an advanced web server, the plant-specific small noncoding RNA interference tool pssRNAit, which can be used to design a pool of small interfering RNAs (siRNAs) for highly effective, specific, and nontoxic gene silencing in plants. In developing this tool, we integrated the transcript dataset of plants, several rules governing gene silencing, and a series of computational models of the biological mechanism of the RNA interference (RNAi) pathway. The designed pool of siRNAs can be used to construct a long double-strand RNA and expressed through virus-induced gene silencing (VIGS) or synthetic transacting siRNA vectors for gene silencing. We demonstrated the performance of pssRNAit by designing and expressing the VIGS constructs to silence Phytoene desaturase (PDS) or a ribosomal protein-encoding gene, RPL10 (QM), in Nicotiana benthamiana We analyzed the expression levels of predicted intended-target and off-target genes using reverse transcription quantitative PCR. We further conducted an RNA-sequencing-based transcriptome analysis to assess genome-wide off-target gene silencing triggered by the fragments that were designed by pssRNAit, targeting different homologous regions of the PDS gene. Our analyses confirmed the high accuracy of siRNA constructs designed using pssRNAit The pssRNAit server, freely available at https://plantgrn.noble.org/pssRNAit/, supports the design of highly effective and specific RNAi, VIGS, or synthetic transacting siRNA constructs for high-throughput functional genomics and trait improvement in >160 plant species.

Schlagwörter:

Gene Expression Regulation, Plant; Genome, Plant/genetics; Oxidoreductases/genetics/metabolism; RNA Interference/physiology; RNA, Small Interfering/genetics; Tobacco/genetics

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.2 exogenous; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Akbar, Sehrish; Wei, Yao; Zhang, Mu-Qing (2022):

RNA Interference: Promising Approach to Combat Plant Viruses.

In: *International journal of molecular sciences* 23 (10). DOI: 10.3390/ijms23105312.

Abstract:

Plant viruses are devastating plant pathogens that severely affect crop yield and quality. Plants have developed multiple lines of defense systems to combat viral infection. Gene silencing/RNA interference is the key defense system in plants that inhibits the virulence and multiplication of pathogens. The general mechanism of RNAi involves (i) the transcription and cleavage of dsRNA into small RNA molecules, such as microRNA (miRNA), or small interfering RNA (siRNA), (ii) the loading of siRNA/miRNA into an RNA Induced Silencing Complex (RISC), (iii) complementary base pairing between siRNA/miRNA with a targeted gene, and (iv) the cleavage or repression of a target gene with an Argonaute (AGO) protein. This natural RNAi pathway could introduce transgenes targeting various viral genes to induce gene silencing. Different RNAi pathways are reported for the artificial silencing of viral genes. These include Host-Induced Gene Silencing (HIGS), Virus-Induced Gene Silencing (VIGS), and Spray-Induced Gene Silencing (SIGS). There are significant limitations in HIGS and VIGS technology, such as lengthy and time-consuming processes, off-target effects, and public concerns regarding genetically modified (GM) transgenic plants. Here, we provide in-depth knowledge regarding SIGS, which efficiently provides RNAi resistance development against targeted genes without the need for GM transgenic plants. We give an overview of the defense system of plants against viral infection, including a detailed mechanism of RNAi, small RNA molecules and their types, and various kinds of RNAi pathways. This review will describe how RNA interference provides the antiviral defense, recent improvements, and their limitations.

Schlagwörter:

Argonaute Proteins/genetics; MicroRNAs; Plant Viruses/genetics; Plants/genetics; RNA Interference; RNA, Double-Stranded/genetics; RNA, Small Interfering/genetics; RNA-Induced Silencing Complex/genetics

Kategorien:

1.6 not applicable; 2.4 viruses; 3.3 not applicable; 4.3 not applicable; 5.2.2 spray (SIGS); 6.9 not applicable; 7.3 not applicable

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Zeitschriftenaufsatz

Allen, Margaret L. (2017):

Comparison of RNAi Sequences in Insect-Resistant Plants to Expressed Sequences of a Beneficial Lady Beetle: A Closer Look at Off-Target Considerations.

In: *Insects* 8 (1). DOI: 10.3390/insects8010027.

Abstract:

Sequences obtained from transcriptomes of the lady beetle Coleomegilla maculata were compared to those designed for incorporation into crops. Searches of the transcriptomes identified sequences as the most likely to be closely similar to the sequences described in RNAi plant incorporated products. Some proposed prime RNAi pest management targets were also used to identify predicted orthologs from C. maculata. The lady beetle sequences were aligned with sequences from corn rootworms and Colorado potato beetles and, as appropriate in the case of targets, regions of similarity were compared with the genetic model organism for beetles, Tribolium castaneum. Some high levels of nucleotide identity were identified, particularly with an actin-derived sequence from Colorado potato beetle. This actin-derived sequence shared identical sequences with the lady beetle and a parasitic wasp.

Schlagwörter:

beneficial organism; genetic pest control; lady beetle; Risk Assessment

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.1.1 Coleomegilla maculata (Lady beetle); 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Arpaia, Salvatore; Christiaens, Olivier; Giddings, Kara; Jones, Huw; Mezzetti, Bruno; Moronta-Barrios, Felix et al. (2020):

Biosafety of GM Crop Plants Expressing dsRNA: Data Requirements and EU Regulatory Considerations.

In: *Frontiers in Plant Science* 11, S. 940. DOI: 10.3389/fpls.2020.00940.

Abstract:

The use of RNA interference (RNAi) enables the silencing of target genes in plants or plant-dwelling organisms, through the production of double stranded RNA (dsRNA) resulting in altered plant

characteristics. Expression of properly synthesized dsRNAs in plants can lead to improved crop quality characteristics or exploit new mechanisms with activity against plant pests and pathogens. Genetically modified (GM) crops exhibiting resistance to viruses or insects via expression of dsRNA have received authorization for cultivation outside Europe. Some products derived from RNAi plants have received a favourable opinion from the European Food Safety Authority (EFSA) for import and processing in the European Union (EU). The authorization process in the EU requires applicants to produce a risk assessment considering food/feed and environmental safety aspects of living organisms or their derived food and feed products. The present paper discusses the main aspects of the safety assessment (comparative assessment, molecular characterization, toxicological assessment, nutritional assessment, gene transfer, interaction with target and non-target organisms) for GM plants expressing dsRNA, according to the guidelines of EFSA. Food/feed safety assessment of products from RNAi plants is expected to be simplified, in the light of the consideration that no novel proteins are produced. Therefore, some of the data requirements for risk assessment do not apply to these cases, and the comparative compositional analysis becomes the main source of evidence for food/feed safety of RNAi plants. During environmental risk assessment, the analysis of dsRNA expression levels of the GM trait, and the data concerning the observable effects on non-target organisms (NTO) will provide the necessary evidence for ensuring safety of species exposed to RNAi plants. Bioinformatics may provide support to risk assessment by selecting target gene sequences with low similarity to the genome of NTOs possibly exposed to dsRNA. The analysis of these topics in risk assessment indicates that the science-based regulatory process in Europe is considered to be applicable to GM RNAi plants, therefore the evaluation of their safety can be effectively conducted without further modifications. Outcomes from the present paper offer suggestions for consideration in future updates of the EFSA Guidance documents on risk assessment of GM organisms.

Schlagwörter:

bioinformatics; biosafety; EU GMO regulation; food safety; non-target organisms; Plants, Genetically Modified; RNAi

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Bally, Julia; Fishilevich, Elane; Doran, Rachel L.; Lee, Karen; Campos, Samanta Bolzan de; German, Marcelo A. et al. (2020):

Plin-amiR, a pre-microRNA-based technology for controlling herbivorous insect pests.

In: *Plant Biotechnol J* 18 (9), S. 1925–1932. DOI: 10.1111/pbi.13352.

Abstract:

The cotton bollworm, Helicoverpa armigera, is a major insect pest for a wide range of agricultural crops. It causes significant yield loss through feeding damage and by increasing the crop's vulnerability to bacterial and fungal infections. Although expression of Bacillus thuringiensis (Bt)

toxins in transgenic crops has been very successful in protecting against insect pests, including H. armigera, field-evolved resistance has occurred in multiple species. To manage resistant populations, new protection strategies must be continuously developed. Trans-kingdom RNA interference (TK-RNAi) is a promising method for controlling herbivorous pests. TK-RNAi is based on delivering dsRNA or hairpin RNA containing essential insect gene sequences to the feeding insect. The ingested molecules are processed by the insect's RNAi machinery and guide it to silence the target genes. Recently, TK-RNAi delivery has been enhanced by expressing the ds- or hpRNAs in the chloroplast. This compartmentalizes the duplexed RNA away from the plant's RNAi machinery, ensuring that it is delivered in an unprocessed form to the insect. Here, we report another alternative approach for delivering precursor anti-insect RNA in plants. Insect pre-microRNA (pre-miR) transcripts were modified to contain artificial microRNAs (amiRs), targeting insect genes, and expressed in transgenic Nicotiana benthamiana plants. These modified pre-miRs remained largely unprocessed in the plants, and H. armigera feeding on leaves from these plants had increased mortality, developmental abnormalities and delayed growth rates. This shows that plant-expressed insect pre-amiRs (plin-amiRs) are a new strategy of protecting plants against herbivorous insects.

Schlagwörter:

Animals; Bacillus thuringiensis; insect control; Insecta; microRNA; MicroRNAs/genetics; Moths/genetics; plant protection; Plants, Genetically Modified/genetics; RNA Interference; trans-kingdom RNAi

Kategorien:

1.5.4 Nicotiana benthamiana; 2.2.1 Helicoverpa armigera (Cotton bollworm/Chickpea pod borer); 3.3 not applicable; 4.1.1.1 miRNA; 4.2.2 post-transcriptional gene regulation; 5.1 endogenous; 5.1.1 stable transgene; 6.6 neuronal system; 6.6.3 termination of nerve impulse transmission; 7.2 Concept demonstration

Zeitschriftenaufsatz

Bally, Julia; McIntyre, Glen J.; Doran, Rachel L.; Lee, Karen; Perez, Alicia; Jung, Hyungtaek et al. (2016):

In-Plant Protection against Helicoverpa armigera by Production of Long hpRNA in Chloroplasts.

In: *Frontiers in Plant Science* 7, S. 1453. DOI: 10.3389/fpls.2016.01453.

Abstract:

Expressing double-stranded RNA (dsRNA) in transgenic plants to silence essential genes within herbivorous pests is referred to as trans-kingdom RNA interference (TK-RNAi) and has emerged as a promising strategy for crop protection. However, the dicing of dsRNA into siRNAs by the plant's intrinsic RNAi machinery may reduce this pesticidal activity. Therefore, genetic constructs, encoding

200 nt duplex-stemmed-hairpin (hp) RNAs, targeting the acetylcholinesterase gene of the cotton bollworm, Helicoverpa armigera, were integrated into either the nuclear or the chloroplast genome of Nicotiana benthamiana. Undiced, full-length hpRNAs accumulated in transplastomic lines of N.

benthamiana and conferred strong protection against H. armigera herbivory while the hpRNAs of nuclear-transformed plants were processed into siRNAs and gave more modest anti-feeding activity. This suggests that there is little or no RNAi machinery or activity in the chloroplast, that hpRNAs produced within this organelle do not enter the cytoplasm, and that oral delivery of chloroplast-packaged intact hpRNA is a more effective means of delivering TK-RNAi than using nuclear encoded hpRNAs. This contrasts with a recently reported correlation between siRNA expression and effectiveness of TK-RNAi targeting the chitinase gene of H. armigera, but is consistent with reports of efficient TK-RNAi by dsRNA generated in chloroplasts by converging promoters flanking a pest gene sequence and from very small (21 nt-stem) hpRNAs resembling artificial miRNAs. Here we demonstrate that hpRNAs, constructed along the conventional design principles of plant RNAi constructs but integrated into the chloroplast genome, are stable and effective over multiple generations, and hold the promise of providing durable pest resistance in crops.

Schlagwörter:

acetylcholinesterase; chloroplast transformation; cotton bollworm (Helicoverpa armigera); hpRNA; insect control; trans-kingdom RNAi

Kategorien:

1.5.4 Nicotiana benthamiana; 2.2.1 Helicoverpa armigera (Cotton bollworm/Chickpea pod borer); 3.3 not applicable; 4.1.1 shRNA; 4.2.2 post-transcriptional gene regulation; 5.1 endogenous; 5.1.1 stable transgene; 5.1.1.1 production in chloroplasts; 6.6 neuronal system; 6.6.3 termination of nerve impulse transmission; 7.1 Concept development

Patentschrift

Baum, James A.; Eads, Brian D.; Kroemer, Jeremy A.; Taylor, Christina Marie (2015):

COMPOSITIONS AND METHODS FOR CONTROLLING INSECT PESTS.

Angemeldet durch MONSANTO TECHNOLOGY LLC [US] am 27.03.2015. Anmeldenr: EP20180188633 20150327. Veröffentlichungsnr: EP3420809 (A1). A01H5/00;A01N63/60;C12N15/11;C12N15/82. Prioriätsdaten: US201461973484P 20140401;EP20150773480 20150327;WO2015US22985 20150327.

Abstract:

Disclosed herein are methods of controlling insect pests which infest crop plants, in particular Spodopterafrugiperda (fall armyworm), Lygus hesperus (western tarnished plant bug), Euschistus heros (neotropical brown stink bug), and Plutella xylostella (diamondback moth), and methods of providing plants resistant to such pests. Also disclosed are polynucleotides and recombinant DNA molecules and constructs useful in such methods, insecticidal compositions such as topical sprays containing insecticidal double-stranded RNAs, and plants with improved resistance to infestation by these insects. Further disclosed are methods of selecting target genes for RNAi-mediated silencing and control of these insect pests.

Kategorien:

2.2 insects

Zeitschriftenaufsatz

Bingsohn, L.; Knorr, E.; Billion, A.; Narva, K. E.; Vilcinskas, A. (2017):

Knockdown of genes in the Toll pathway reveals new lethal RNA interference targets for insect pest control.

In: *Insect molecular biology* 26 (1), S. 92–102. DOI: 10.1111/imb.12273.

Abstract:

RNA interference (RNAi) is a promising alternative strategy for ecologically friendly pest management. However, the identification of RNAi candidate genes is challenging owing to the absence of laboratory strains and the seasonality of most pest species. Tribolium castaneum is a well-established model, with a strong and robust RNAi response, which can be used as a high-throughput screening platform to identify potential RNAi target genes. Recently, the cactus gene was identified as a sensitive RNAi target for pest control. To explore whether the spectrum of promising RNAi targets can be expanded beyond those found by random large-scale screening, to encompass others identified using targeted knowledge-based approaches, we constructed a Cactus interaction network. We tested nine genes in this network and found that the delivery of double-stranded RNA corresponding to fusilli and cactin showed lethal effects. The silencing of cactin resulted in 100% lethality at every developmental stage from the larva to the adult. The knockdown of pelle, Dorsal-related immunity factor and short gastrulation reduced or even prevented egg hatching in the next generation. The combination of such targets with lethal and parental RNAi effects can now be tested against different pest species in field studies.

Schlagwörter:

Animals; DNA-Binding Proteins/genetics; Drosophila Proteins/genetics; Female; Gene Regulatory Networks; Genes, Insect; Heterogeneous-Nuclear Ribonucleoprotein Group F-H/genetics; insect control; Male; Phosphoproteins/genetics; Reproduction; RNA Interference; Tribolium/genetics

Kategorien:

1.6 not applicable; 2.2.12 Tribolium castaneum (Red flour beetle); 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.1.17 cell proliferation, mitosis; 6.2.5.2 embryonic dorsoventral formation; 6.5 immune system; 6.5.3 defense system against fungal and bacterial infections; 6.5.4 Toll-like pathway; 7.1 Concept development

Zeitschriftenaufsatz

Bocos-Asenjo, Irene Teresa; Niño-Sánchez, Jonatan; Ginésy, Mireille; Diez, Julio Javier (2022):

New Insights on the Integrated Management of Plant Diseases by RNA Strategies: Mycoviruses and RNA Interference.

In: *International journal of molecular sciences* 23 (16). DOI: 10.3390/ijms23169236.

Abstract:

RNA-based strategies for plant disease management offer an attractive alternative to agrochemicals that negatively impact human and ecosystem health and lead to pathogen resistance. There has been recent interest in using mycoviruses for fungal disease control after it was discovered that some cause hypovirulence in fungal pathogens, which refers to a decline in the ability of a pathogen to cause disease. Cryphonectria parasitica, the causal agent of chestnut blight, has set an ideal model of management through the release of hypovirulent strains. However, mycovirus-based management of plant diseases is still restricted by limited approaches to search for viruses causing hypovirulence and the lack of protocols allowing effective and systemic virus infection in pathogens. RNA interference (RNAi), the eukaryotic cell system that recognizes RNA sequences and specifically degrades them, represents a promising. RNA-based disease management method. The natural occurrence of cross-kingdom RNAi provides a basis for host-induced gene silencing, while the ability of most pathogens to uptake exogenous small RNAs enables the use of spray-induced gene silencing techniques. This review describes the mechanisms behind and the potential of two RNA-based strategies, mycoviruses and RNAi, for plant disease management. Successful applications are discussed, as well as the research gaps and limitations that remain to be addressed.

Schlagwörter:

Ecosystem; Fungal Viruses/genetics; Humans; Plant Diseases/genetics/microbiology/therapy; Plants/genetics; RNA; RNA Interference; Viruses/genetics

Kategorien:

1.6 not applicable; 2.1 fungi; 3.3 not applicable; 5.2.1 VIGS; 5.2.2 spray (SIGS); 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Bowen, Joanna K.; Brummell, David A.; Gapper, Nigel E. (2022):

Biotechnological approaches for reducing fruit losses caused by pathogenic infection.

In: *Current opinion in biotechnology* 78, S. 102795. DOI: 10.1016/j.copbio.2022.102795.

Abstract:

Fruit loss due to disease occurs in both the field and postharvest. Knowledge of host immune responses and pathogen virulence is enabling the formulation of increasingly sophisticated strategies for disease control. Traditional genetic modification, typically involving overexpression of genes involved in pathogen perception and defence responses, is beginning to be superseded by CRISPR-Cas9 manipulation of host susceptibility targets. Moreover, the refinement of RNA interference (RNAi) strategies, including spray-induced gene silencing (SIGS), is allowing more nuanced control options. These latter approaches have the advantage over earlier technologies in that either they do not result in the generation of genetically modified organisms (RNAi-based SIGS), or the genetic manipulation used leaves no trace of introduced genetic material (gene editing). Thus, these strategies may be more widely acceptable for deployment for future disease control.

Schlagwörter:

Fruit/genetics; gene editing; RNA Interference; Virulence/genetics

Kategorien:

1.6 not applicable; 2.4 viruses; 3.3 not applicable; 4.3 not applicable; 5.2 exogenous; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Cagliari, Deise; Dias, Naymã P.; Galdeano, Diogo Manzano; Dos Santos, Ericmar Ávila; Smagghe, Guy; Zotti, Moisés João (2019):

Management of Pest Insects and Plant Diseases by Non-Transformative RNAi.

In: *Frontiers in Plant Science* 10, S. 1319. DOI: 10.3389/fpls.2019.01319.

Abstract:

Since the discovery of RNA interference (RNAi), scientists have made significant progress towards the development of this unique technology for crop protection. The RNAi mechanism works at the mRNA level by exploiting a sequence-dependent mode of action with high target specificity due to the design of complementary dsRNA molecules, allowing growers to target pests more precisely compared to conventional agrochemicals. The delivery of RNAi through transgenic plants is now a reality with some products currently in the market. Conversely, it is also expected that more RNA-based products reach the market as non-transformative alternatives. For instance, topically applied dsRNA/siRNA (SIGS - Spray Induced Gene Silencing) has attracted attention due to its feasibility and low cost compared to transgenic plants. Once on the leaf surface, dsRNAs can move directly to target pest cells (e.g., insects or pathogens) or can be taken up indirectly by plant cells to then be transferred into the pest cells. Water-soluble formulations containing pesticidal dsRNA provide alternatives, especially in some cases where plant transformation is not possible or takes years and cost millions to be developed (e.g., perennial crops). The ever-growing understanding of the RNAi mechanism and its limitations has allowed scientists to develop non-transgenic approaches such as trunk injection, soaking, and irrigation. While the technology has been considered promising for pest management, some issues such as RNAi efficiency, dsRNA degradation, environmental risk assessments, and resistance evolution still need to be addressed. Here, our main goal is to review some possible strategies for non-transgenic delivery systems, addressing important issues related to the use of this technology.

Schlagwörter:

gene silencing; non-transgenic RNA; pest insect; Plant Diseases; RNA-based products; RNAi (RNA interference)

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.2 exogenous; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Cai, Qiang; He, Baoye; Kogel, Karl-Heinz; Jin, Hailing (2018):

Cross-kingdom RNA trafficking and environmental RNAi-nature's blueprint for modern crop protection strategies.

In: *Current opinion in microbiology* 46, S. 58–64. DOI: 10.1016/j.mib.2018.02.003.

Abstract:

In plants, small RNA (sRNA)-mediated RNA interference (RNAi) is critical for regulating host immunity against bacteria, fungi, oomycetes, viruses, and pests. Similarly, sRNAs from pathogens and pests also play an important role in modulating their virulence. Strikingly, recent evidence supports that some sRNAs can travel between interacting organisms and induce gene silencing in the counter party, a mechanism termed cross-kingdom RNAi. Exploiting this new knowledge, host-induced gene silencing (HIGS) by transgenic expression of pathogen gene-targeting double-stranded (ds)RNA has the potential to become an important disease-control method. To circumvent transgenic approaches, direct application of dsRNAs or sRNAs (environmental RNAi) onto host plants or post-harvest products leads to silencing of the target microbe/pest gene (referred to spray-induced gene silencing, SIGS) and confers efficient disease control. This review summarizes the current understanding of cross-kingdom RNA trafficking and environmental RNAi and how these findings can be developed into novel effective strategies to fight diseases caused by microbial pathogens and pests.

Schlagwörter:

Fungi/genetics/physiology; Plant Diseases/genetics/immunology/microbiology; Plants/genetics/immunology/microbiology; RNA Interference; RNA, Plant/genetics/immunology; RNA, Small Interfering/genetics/immunology

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Çalışır, Kübra; Krczal, Gabi; Uslu, Veli Vural (2022):

Small RNA-seq dataset of wild type and 16C Nicotiana benthamiana leaves sprayed with naked dsRNA using the high-pressure spraying technique.

In: *Data in brief* 45, S. 108706. DOI: 10.1016/j.dib.2022.108706.

Abstract:

Double-stranded RNA (dsRNA) applications have emerged as promising alternatives to chemical plant pesticides. It has been proposed that the protective effect of dsRNA is mediated by the RNA interference (RNAi) mechanism. Small RNAs (sRNAs) are one of the landmarks of RNAi mechanisms. Two classes of sRNAs appear upon RNAi, triggered by dsRNA: The cleavage products of the dsRNA mapping directly to the dsRNA sequence and the transitive sRNAs mapping to the target transcript outside of the dsRNA sequence. Therefore, the sRNA-seq data obtained from dsRNA-treated plants have been exclusively analysed in the context of the target genes and the outcome has been considered essential to evaluate the underlying mechanism of dsRNA mediated plant protection. Using high-pressure spraying technology (HPST), we have applied a GFP targeting 139bp-long dsRNA on wild type (WT) and GFP expressing (16C) Nicotiana benthamiana plants in biological triplicates. As a control, we applied water with HPST on 16C N. benthamiana. We have acquired sRNA-seq data on the treated and control leaves 5 days post spraying. In this dataset, we have expanded our sRNA-seq analysis from the target GFP transgene sequence to the whole transcriptome of N. benthamiana to provide the community with a resource for the small RNA landscape after high-pressure spraying in 16C and WT samples. Furthermore, we have provided a comparison of sRNA landscape between WT and 16C lines.

Schlagwörter:

dsRNA; exogenous dsRNAs; post-transcriptpional gene silencing (PTGS); RNAi (RNA interference); SIGS (spray-induced gene silencing)

Kategorien:

1.5.4 Nicotiana benthamiana; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable;
5.2 exogenous; 5.2.2 spray (SIGS); 6.9 not applicable; 7.1 Concept development

Zeitschriftenaufsatz

Camargo, Roberto A.; Barbosa, Guilherme O.; Possignolo, Isabella Presotto; Peres, Lazaro E. P.; Lam, Eric; Lima, Joni E. et al. (2016):

RNA interference as a gene silencing tool to control Tuta absoluta in tomato (Solanum lycopersicum).

In: *PeerJ* 4, e2673. DOI: 10.7717/peerj.2673.

Abstract:

RNA interference (RNAi), a gene-silencing mechanism that involves providing double-stranded RNA molecules that match a specific target gene sequence, is now widely used in functional genetic studies. The potential application of RNAi-mediated control of agricultural insect pests has rapidly become evident. The production of transgenic plants expressing dsRNA molecules that target essential insect genes could provide a means of specific gene silencing in larvae that feed on these plants, resulting in larval phenotypes that range from loss of appetite to death. In this report, we show that the tomato leafminer (Tuta absoluta), a major threat to commercial tomato production, can be targeted by RNAi. We selected two target genes (Vacuolar ATPase-A and Arginine kinase) based on the RNAi response reported for these genes in other pest species. In view of the lack of an artificial diet for T. absoluta, we used two approaches to deliver dsRNA into tomato leaflets. The first approach was based on the uptake of dsRNA by leaflets and the second was based on "in planta-induced transient gene silencing" (PITGS), a well-established method for silencing plant genes, used here for the first time to deliver in planta-transcribed dsRNA to target insect genes. Tuta absoluta larvae that fed on leaves containing dsRNA of the target genes showed an 60% reduction in target gene transcript accumulation, an increase in larval mortality and less leaf damage. We then generated transgenic 'Micro-Tom' tomato plants that expressed hairpin sequences for both genes and observed a reduction in foliar damage by T. absoluta in these plants. Our results demonstrate the feasibility of RNAi as an alternative method for controlling this critical tomato pest.

Schlagwörter:

agro-infiltration; dsRNA delivery; dsRNA uptake; gene silencing; micro-tom; Pest Control; RNAi (RNA interference); transgene; Tuta absoluta

Kategorien:

1.4.8 Solanum lycopersium (tomato); 2.2.31 Tuta absoluta (Tomato leafminer); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.1 endogenous; 5.1.2 transient transgene; 5.2 exogenous; 6.1.16 cell ion uptake; 6.3 energy metabolism; 7.2 Concept demonstration

Zeitschriftenaufsatz

Chaitanya, B. N.; Asokan, R.; Sita, T.; Rebijith, K. B.; Ram Kumar, P.; Krishna Kumar, N. K. (2017):

Silencing of JHEH and EcR genes of Plutella xylostella (Lepidoptera: Plutellidae) through double stranded RNA oral delivery.

In: *Journal of Asia-Pacific Entomology* 20 (2), S. 637–643. DOI: 10.1016/j.aspen.2017.03.020.

Abstract:

Plutella xylostella (L), Diamondback moth (DBM), is a notorious pest disrupting cruciferous crops across the world. Overuse of chemically synthesized insecticides as well as application of bio-pesticide like Bacillus thuringiensis has gained progressive resistance against them, resulting in DBM management failures. To overcome this, a more potential, non-chemical and safe technology like Ribonucleic acid interference (RNAi) is to be implemented. RNAi, is a reverse genetic tool with high sequence specificity, elicited by double-stranded RNA (dsRNA) for silencing the target genes. The objective of this study was to silence two candidate genes from DBM viz., Juvenile Hormone Epoxide hydrolase (PxJHEH) and Ecdysteroid receptor (PxEcR) involved in regulating metamorphosis and molting respectively. In this regard, we have custom designed the dsRNAs (500 bp) by cloning and sequencing the candidate genes and delivered it orally (non-invasive mode). Further, we have assessed the extent of down regulation of target genes with five concentrations of dsRNA (1, 2, 5, 10 and 20 Mil) at four time points (24, 48, 72 and 96 h). The extent of gene silencing and mortality recorded for both genes were proportional to the dsRNA concentration. Our study projects that PxJHEH and PxEcR genes would be good target for dsRNA mediated gene silencing in management of insect pests such as DBM in near future.

Schlagwörter:

ecdysteroid receptor; juvenile hormone epoxide hydrolase; Plutella xylostella; RNAi (RNA interference); RNAi-based pest management

Kategorien:

1.6 not applicable; 2.2.11 Plutella xylostella (Diamondback moth); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2 exogenous; 6.2.4 molting; 6.2.5.1 metamorphosis; 7.1 Concept development

Zeitschriftenaufsatz

Chen, Wanxin; Kastner, Christine; Nowara, Daniela; Oliveira-Garcia, Ely; Rutten, Twan; Zhao, Yusheng et al. (2016):

Host-induced silencing of Fusarium culmorum genes protects wheat from infection.

In: *Journal of experimental botany* 67 (17), S. 4979–4991. DOI: 10.1093/jxb/erw263.

Abstract:

Plants producing antisense or double-stranded RNA molecules that target specific genes of eukaryotic pests or pathogens can become protected from their attack. This beneficial effect was also reported for plant-fungus interactions and is believed to reflect uptake of the RNAs by the fungus via an as yet unknown mechanism, followed by target gene silencing. Here we report that wheat plants pre-infected with Barley stripe mosaic virus (BSMV) strains containing antisense sequences against target genes of the Fusarium head blight (FHB) fungus F. culmorum caused a reduction of corresponding transcript levels in the pathogen and reduced disease symptoms. Stable transgenic wheat plants carrying an RNAi hairpin construct against the β-1, 3-glucan synthase gene FcGls1 of F. culmorum or a triple combination of FcGls1 with two additional, pre-tested target genes also showed enhanced FHB resistance in leaf and spike inoculation assays under greenhouse and near-field conditions, respectively. Microscopic evaluation of F. culmorum development in plants transiently or stably expressing FcGls1 silencing constructs revealed aberrant, swollen fungal hyphae, indicating severe hyphal cell wall defects. The results lead us to propose host-induced gene silencing (HIGS) as a plant protection approach that may also be applicable to highly FHB-susceptible wheat genotypes.

Schlagwörter:

Disease Resistance/physiology; Fusarium/pathogenicity; Gene Silencing/physiology; Genes, Bacterial/genetics; Host-Pathogen Interactions; Plant Diseases/microbiology; Plant Leaves/microbiology; Plants, Genetically Modified; Real-Time Polymerase Chain Reaction; Reverse Transcriptase Polymerase Chain Reaction; RNA, Antisense/genetics/physiology; Triticum/metabolism/microbiology

Kategorien:

1.1.3 Triticum aestivum (wheat); 2.1.5 Fusarium culmorum (Fusarium head blight fungus (FHB)); 3.3 not applicable; 4.1.1 shRNA; 5.1 endogenous; 5.1.1 stable transgene; 5.2.1 VIGS; 6.1.12 cytosceleton; 6.1.17 cell proliferation, mitosis; 6.2.3 chitin synthesis; 6.3 energy metabolism; 6.7.8 infection mechanisms (virulence factors); 7.2 Concept demonstration

Zeitschriftenaufsatz

Chen, Xu; Li, Guo-Qing; Wan, Pin-Jun; Fu, Qiang (2017):

Efficient RNA interference for three neuronally-expressed genes in Nilaparvata lugens (Stål) (Hemiptera: Delphacidae).

In: *Journal of Asia-Pacific Entomology* 20 (2), S. 513–519. DOI: 10.1016/j.aspen.2017.03.009.

Abstract:

In the brown planthopper Nilaparvata lugens, introduction of double-stranded RNA (dsRNA) successfully knocks down genes transcribed in several types of tissues. However, whether RNA interference (RNAi) can effectively reduce the transcripts of neuronally-expressed genes remains undetermined. In the present paper, we selected three neuronally-expressed genes respectively encoding prothoracicotropic hormone (NIPTTH), pigment-dispersing factor (NIPDF) and short neuropeptide F (NIsNPF). The transcripts of the three genes were detectable throughout all tested developmental stages, and were mainly restricted to the nervous system of N. lugens. We introduced dsRNA originated from each of the three neuronally-expressed genes at a dose of 300 ng into the nymphal body cavity by microinjection. The target mRNA was successfully knocked down. Moreover, silencing of NIPTTFI downregulated the transcripts of two ecdysteroidogenesis genes (NIPHM and NIDIB), and surpressed the expression of an ecdysone-response genes (NIFTZ-FI). Furthermore, RNAi of NIPTTH caused typical 20-hydroxyecdysone deficient phenotypes: nymphal development was delayed and nymphal lethality occurred. In addition, injection of a series of dsNIsDPF solutions at the doses of 300, GO, 12, 2.4 and 0.5 ng into the fourthinstar nymphs decreased its target transcript in a dose-dependent manner: the mRNA levels reduced by 94%, 86%, 57%, 33% and 29%, respectively. Therefore, delivery of dsRNA by microinjection can effectively repress the transcripts of the neuronally-expressed genes in N. lugens. (C) 2017 Published by Elsevier B.V. on behalf of Korean Society of Applied Entomology, Taiwan Entomological Society and Malaysian Plant Protection Society.

Schlagwörter:

efficiency; neuronally-expressed gene; Nilaparvata lugens; RNAi (RNA interference)

Kategorien:

1.6 not applicable; 2.2.14 Nilaparvata lugens (Brown planthopper); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2.1 mRNA degradation; 5.2 exogenous; 5.2.9 microinjection; 6.2.4 molting; 6.6.1 neuro-endocrine communication; 7.1 Concept development

Zeitschriftenaufsatz

CHEN, Tai-yu; HOU, Ji-xiang; LIN, Yong-jun (2016):

Transcriptome datasets supply basic gene information for RNAi pest management and gene functional studies in Nephotettix cincticeps (Uhler).

In: *Journal of Integrative Agriculture* 15 (4), S. 840–847. DOI: 10.1016/S2095-3119(15)61133-9.

Abstract:

RNA interference (RNAi) technology has the potential to be used in pest management in crop production. Here, the

transcriptome of Nephotettix cincticeps (Uhler) was deeply sequenced to investigate the systematic RNAi mechanism

and candidate genes for dsRNA feeding. In our datasets, a total of 81 225 transcripts were obtained with the length from

150 bp to about 4.2 kb. Almost all the genes related to the RNAi core pathway were proved to be present in N. cincticeps

transcriptome. Two transcripts that respectively encode a systemic interference defective (SID) were identified in our da-

tabase, indicating that the systematic RNAi pathway can function effectively in N. cincticeps. Our datasets not only supply

basic gene information for the studies of gene expression and functions in N. cincticeps, such as the control genes for gene

expression analysis, but also provide candidate genes for RNAi pest management, such as the genes that encode P450

monooxygenase, V-ATPase and chitin synthase.

Schlagwörter:

house-keeping genes; Nephotettix cincticeps; RNAi; Transcriptome

Kategorien:

1.6 not applicable; 2.2.16 Nephotettix cincticeps (Rice green leafhopper); 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.2.3 chitin synthesis; 6.3 energy metabolism; 6.3.8 metabolism; 7.1 Concept development

Zeitschriftenaufsatz

Christiaens, Olivier; Niu, Jinzhi; Nji Tizi Taning, Clauvis (2020):

RNAi in Insects: A Revolution in Fundamental Research and Pest Control Applications.

In: *Insects* 11 (7). DOI: 10.3390/insects11070415.

Abstract:

In this editorial for the Special Issue on 'RNAi in insect pest control', three important applications of RNA interference (RNAi) in insects are briefly discussed and linked to the different studies published in this Special Issue. The discovery of the RNAi mechanism revolutionized entomological research, as it presented researchers with a tool to knock down genes, which is easily applicable in a wide range of insect species. Furthermore, RNAi also provides crop protection with a novel and promising pest control mode-of-action. The sequence-dependent nature allows RNAi-based control strategies to be

highly species selective and the active molecule, a natural biological molecule known as double-stranded RNA (dsRNA), has a short environmental persistence. However, more research is needed to investigate different cellular and physiological barriers, such as cellular uptake and dsRNA degradation in the digestive system in insects, in order to provide efficient control methods against a wide range of insect pest species. Finally, the RNAi pathway is an important part of the innate antiviral immune defence of insects, and could even lead to applications targeting viruses in beneficial insects such as honeybees in the future.

Schlagwörter:

antiviral immunity; crop protection; gene silencing; loss-of-function analysis; RNAi

Kategorien:

1.6 not applicable; 2.2 insects; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Christiaens, Olivier; Smagghe, Guy (2014):

The challenge of RNAi-mediated control of hemipterans.

In: *Current opinion in insect science* 6, S. 15–21. DOI: 10.1016/j.cois.2014.09.012.

Abstract:

The post-transcriptional gene silencing mechanism RNA interference (RNAi) has potential as a crop protection strategy against important pest insects. Here we focus on Hemiptera pests, comprising some of the most devastating pest organisms as aphids, whiteflies, psyllids, bedbugs and kissing bugs. At first, a state-of-the-art overview is provided of the progress in RNAi in Hemiptera, as well as on the challenges when developing new RNAi-based pest control strategies against hemipteran pests, such as the delivery of dsRNA and degradation in the insect body. We also discuss the variability in RNAi efficiency as observed between species and experiments, and the factors potentially responsible for this phenomenon.

Kategorien:

1.6 not applicable; 2.2.32 Hemiptera; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Christiaens, Olivier; Whyard, Steve; Vélez, Ana M.; Smagghe, Guy (2020):

Double-Stranded RNA Technology to Control Insect Pests: Current Status and Challenges.

In: *Frontiers in Plant Science* 11, S. 451. DOI: 10.3389/fpls.2020.00451.

Abstract:

Exploiting the RNA interference (RNAi) gene mechanism to silence essential genes in pest insects, leading to toxic effects, has surfaced as a promising new control strategy in the past decade. While the first commercial RNAi-based products are currently coming to market, the application against a wide range of insect species is still hindered by a number of challenges. In this review, we discuss the current status of these RNAi-based products and the different delivery strategies by which insects can be targeted by the RNAi-triggering double-stranded RNA (dsRNA) molecules. Furthermore, this review also addresses a number of physiological and cellular barriers, which can lead to decreased RNAi efficacy in insects. Finally, novel non-transgenic delivery technologies, such as polymer or liposomic nanoparticles, peptide-based delivery vehicles and viral-like particles, are also discussed, as these could overcome these barriers and lead to effective RNAi-based pest control.

Schlagwörter:

dsRNA; HIGS (host-induced gene silencing); RNAi (RNA interference); RNAi-based pest management; SIGS (spray-induced gene silencing); virus-induced gene silencing (VIGS)

Kategorien:

1.6 not applicable; 2.2 insects; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Patentschrift

Coelho, Pedro; Wheeler, Christopher (2017):

IMPROVED INSECT CONTROL STRATEGIES UTILIZING PHEROMONES AND RNAI.

Angemeldet durch PROVIVI INC [US] am 26.05.2017. Anmeldenr: US201716304762 20170526. Veröffentlichungsnr: US2019343122 (A1).
A01N25/00;A01N27/00;A01N49/00;A01N57/16;C12N15/82. Prioriätsdaten: US201662342807P 20160527;US201716304762 20170526;WO2017US34697 20170526.

Abstract:

Systems and methods of preventing or reducing crop damage from pests are provided. In one embodiment, the method comprises: a) applying a mating disruption tactic to a field plot; and b) disrupting expression of one or more target genes in one or more pests, wherein crop damage is reduced in the field plot. In another embodiment, the method comprises applying an attract-and-kill tactic to a field plot, wherein said attract-and-kill tactic comprises: a) applying one or more semiochemicals or factors; and b) disrupting expression of one or more target genes in one or more pests, wherein said disruption is capable of killing the one or more pests, wherein crop damage is reduced in the field plot.

Kategorien:

2.2 insects; 5.2.8 pheromone-coupled

Zeitschriftenaufsatz

Cooper, Anastasia M. W.; Song, Huifang; Shi, Xuekai; Yu, Zhitao; Lorenzen, Marcé; Silver, Kristopher et al. (2020):

Molecular Characterizations of Double-Stranded RNA Degrading Nuclease Genes from Ostrinia nubilalis.

In: *Insects* 11 (10). DOI: 10.3390/insects11100652.

Abstract:

Variable RNA interference (RNAi) efficiencies limit RNAi-based pest management strategies for many pests. Previous efforts to understand mechanisms contributing to low RNAi efficiency indicate that double-stranded RNA (dsRNA) is degraded in the European corn borer (ECB), Ostrinia nubilalis, due to nuclease activity. To investigate the contribution of dsRNA-degrading endonucleases (dsRNases) and lepidopteran-specific RNAi efficiency-related nucleases (REases) to dsRNA instability and low RNAi efficiency in ECB, five complementary DNAs putatively encoding four dsRNases (OndsRNase1, 2, 3, and 4) and one REase (OnREase) were sequenced. Characterization of these transcripts revealed that substrate specificity might vary among the four dsRNases due to different amino acid combinations in the substrate-binding sites. Gene expression analysis indicated that OndsRNase2 and OnREase were highly expressed in the larval gut, and OndsRNase1 showed the highest expression in hemolymph, especially in older developmental stages. Transcript level analysis after dsRNA exposure revealed that expression of OnREase rapidly increased upon dsRNA ingestion or injection, whereas OndsRNase4 expression only increased after long-term ingestion of dsRNA. While the biological function of these nucleases remains to be verified, our results suggest that OnREase and OndsRNase2, and OndsRNase1 and OndsRNase4 may be responsible for degradation of dsRNAs in the ECB gut and hemolymph, respectively, thereby contributing to low RNAi efficiency.

Schlagwörter:

dsRNase; European corn borer; gene expression; REase; RNAi efficiency; substrate specificity

Kategorien:

1.6 not applicable; 2.2.7 Ostrinia nubilalis (European corn borer); 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Cooper, Anastasia M. W.; Song, Huifang; Shi, Xuekai; Yu, Zhitao; Lorenzen, Marcé; Silver, Kristopher et al. (2021):

Characterization, expression patterns, and transcriptional responses of three core RNA interference pathway genes from Ostrinia nubilalis.

In: *Journal of insect physiology* 129, S. 104181. DOI: 10.1016/j.jinsphys.2020.104181.

Abstract:

RNA interference (RNAi) is commonly used in the laboratory to analyze gene function, and RNAi-based pest management strategies are now being employed. Unfortunately, RNAi is hindered by inefficient and highly-variable results when different insects are targeted, especially lepidopterans, such as the European corn borer (ECB), Ostrinia nubilalis (Lepidoptera: Crambidae). Previous efforts to achieve RNAi-mediated gene suppression in ECB revealed low RNAi efficiency with both double-stranded RNA (dsRNA) injection and ingestion. One mechanism that can affect RNAi efficiency in insects is the expression and function of core RNAi pathway genes, such as those encoding Argonaut 2 (Ago2), Dicer 2 (Dcr2), and a dsRNA binding protein (R2D2). To determine if deficiencies in these core RNAi pathway genes contribute to low RNAi efficiency in ECB, full-length complementary DNAs encoding OnAgo2, OnDcr2, and OnR2D2 were cloned, sequenced, and characterized. A comparison of domain architecture suggested that all three predicted proteins contained the necessary domains to function. However, a comparison of evolutionary distances revealed potentially important variations in the first RNase III domain of OnDcr2, the double-stranded RNA binding domains of OnR2D2, and both the PAZ and PIWI domains of OnAgo2, which may indicate functional differences in enzymatic activity between species. Expression analysis indicated that transcripts for all three genes were expressed in all developmental stages and tissues investigated. Interestingly, the introduction of non-target dsRNA into ECB second-instar larvae via microinjection did not affect OnAgo2, OnDcr2, or OnR2D2 expression. In contrast, ingestion of the same dsRNAs resulted in upregulation of OnDcr2 but downregulation of OnR2D2. The unexpected transcriptional responses of the core machinery and the divergence in amino-acid sequence between specific domains in each core RNAi protein may possibly contribute to low RNAi efficiency in ECB. Understanding the contributions of different RNAi pathway components is critical to adapting this technology for use in controlling lepidopteran pests that exhibit low RNAi efficiency.

Schlagwörter:

Animals; Argonaute Proteins/genetics; Genes, Insect/drug effects; Insect Control/methods; Moths/genetics/metabolism; RNA Helicases/genetics; RNA Interference; RNA, Small Interfering/pharmacology; RNA-Binding Proteins/genetics; RNAi Therapeutics

Kategorien:

1.6 not applicable; 2.2.7 Ostrinia nubilalis (European corn borer); 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Cooper, Anastasia Mw; Silver, Kristopher; Zhang, Jianzhen; Park, Yoonseong; Zhu, Kun Yan (2019):

Molecular mechanisms influencing efficiency of RNA interference in insects.

In: *Pest management science* 75 (1), S. 18–28. DOI: 10.1002/ps.5126.

Abstract:

RNA interference (RNAi) is an endogenous, sequence-specific gene-silencing mechanism elicited by small RNA molecules. RNAi is a powerful reverse genetic tool, and is currently being utilized for managing insects and viruses. Widespread implementation of RNAi-based pest management strategies is currently hindered by inefficient and highly variable results when different insect species, strains, developmental stages, tissues, and genes are targeted. Mechanistic studies have

shown that double-stranded ribonucleases (dsRNases), endosomal entrapment, deficient function of the core machinery, and inadequate immune stimulation contribute to limited RNAi efficiency. However, a comprehensive understanding of the molecular mechanisms limiting RNAi efficiency remains elusive. Recent advances in dsRNA stability in physiological tissues, dsRNA internalization into cells, the composition and function of the core RNAi machinery, as well as small-interfering RNA/double-stranded RNA amplification and spreading mechanisms are reviewed to establish a global understanding of the obstacles impeding wider understanding of RNAi mechanisms in insects.

Schlagwörter:

Animals; Insect Proteins/genetics; Insecta/genetics; RNA Interference; RNA, Double-Stranded/genetics

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

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Zeitschriftenaufsatz

Dalakouras, Athanasios; Jarausch, Wolfgang; Buchholz, Guenther; Bassler, Alexandra; Braun, Mario; Manthey, Thorsten et al. (2018):

Delivery of Hairpin RNAs and Small RNAs Into Woody and Herbaceous Plants by Trunk Injection and Petiole Absorption.

In: *Frontiers in Plant Science* 9, S. 1253. DOI: 10.3389/fpls.2018.01253.

Abstract:

Since its discovery, RNA interference has been widely used in crop protection. Recently, transgene-free procedures that were based on exogenous application of RNA molecules having the capacity to trigger RNAi in planta have been reported. Yet, efficient delivery of such RNA molecules to plants and particularly to trees poses major technical challenges. Here, we describe simple methods for efficient delivery of hairpin RNAs (hpRNAs) and small interfering RNAs (siRNAs) to Malus domestica, Vitis vinifera, and Nicotiana benthamiana that are based on trunk injection and/or petiole absorption. The applied RNA molecules were efficiently taken up and systemically transported. In apical leaves, the RNA was already detectable 1 day post-application (dpa) and could be detected at least up to 10 dpa, depending on the method of application. Confocal microscopy revealed that the uptaken and systemically transported RNA molecules were strictly restricted to the xylem and apoplast which may illustrate why the applied hpRNAs were not processed into siRNAs by plant DICER-LIKE (DCL) endonucleases. These innovative methods may have great impact in pest management against chewing and/or xylem sap-feeding vectors and eukaryotic pathogens that reside in the xylem.

Schlagwörter:

dsRNA; Malus domestica; petiola; RNAi; siRNA; tobacco (Nicotiana benthamiana); trunk; Vitis vinifera

Kategorien:

1.3.1 Malus domestica; 1.3.2 Vitis vinifera (grape); 1.5.4 Nicotiana benthamiana; 2.6.1 xylem-feeding organisms; 3.3 not applicable; 4.1.1 shRNA; 4.2.2 post-transcriptional gene regulation; 4.2.2.1 mRNA degradation; 5.2 exogenous; 5.2.7 plant injection; 6.9 not applicable; 7.1 Concept development

Zeitschriftenaufsatz

Das, Joy; Kumar, Rakesh; Shah, Vivek; Sharma, Ashwani Kumar (2022):

Functional characterization of chitin synthesis pathway genes, HaAGM and HaUAP, reveal their crucial roles in ecdysis and survival of Helicoverpa armigera (Hübner).

In: *Pesticide biochemistry and physiology* 188, S. 105273. DOI: 10.1016/j.pestbp.2022.105273.

Abstract:

The chitin metabolic pathway is one of the most lucrative targets for designing pest management regimes. Inhibition of the chitin synthesis pathway causes detrimental effects on the normal growth and development of insects. Phospho-N-acetylglucosamine mutase (AGM) and UDP-N-acetylglucosamine pyrophosphorylase (UAP) are two key chitin biosynthesis enzymes in insects including Helicoverpa armigera, a pest of global significance. In the present study, we have identified, cloned and recombinantly expressed AGM and UAP from H. armigera (HaAGM and HaUAP). Biochemical characterization of recombinant HaAGM and HaUAP exhibited high affinities for their natural substrates N-acetyl glucosamine-6-phosphate (Km 38.72 ± 2.41) and N-acetyl glucosamine-1-phosphate (Km 3.66 ± 0.13), respectively. In the coupled enzyme-catalytic assay, HaAGM and HaUAP yielded the end-products, inorganic pyrophosphate and UDP-GlcNAc, confirming their active participation in the chitin synthesis pathway of H. armigera. Gene expression profiling revealed that HaAGM and HaUAP genes were expressed in all developmental stages and key tissues. These genes also showed substantial responses towards the moulting hormone 20-hydroxyecdysone and chitin biosynthesis inhibitor, novaluron. Remarkably, the RNAi-mediated knockdown of either HaAGM or HaUAP led to severe developmental deformities and significant mortality ranging from 65.61 to 72.54%. Overall findings suggest that HaAGM and HaUAP play crucial roles in the ecdysis and survival of H. armigera. Further, these genes could serve as potential targets for designing pest management strategies for H. armigera.

Schlagwörter:

Animals; Chitin; Ecdysterone/pharmacology; Glucosamine; Molting/genetics; Moths/genetics

Kategorien:

1.6 not applicable; 2.2.1 Helicoverpa armigera (Cotton bollworm/Chickpea pod borer); 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.2.3 chitin synthesis; 6.2.4 molting; 7.1 Concept development

Zeitschriftenaufsatz

Das, Protiva Rani; Sherif, Sherif M. (2020):

Application of Exogenous dsRNAs-induced RNAi in Agriculture: Challenges and Triumphs.

In: *Frontiers in Plant Science* 11, S. 946. DOI: 10.3389/fpls.2020.00946.

Abstract:

In recent years, RNA interference (RNAi) machinery has widely been explored by plant biologists for its potential applications in disease management, plant development, and germplasm improvement. RNAi-based technologies have mainly been applied in the form of transgenic plant generation and host-induced-gene-silencing (HIGS). However, the approval of RNAi-based transgenic plants has always been challenging due to the proclaimed concerns surrounding their impacts on human health and the environment. Lately, exogenous applications of double-stranded RNAs (dsRNAs), short interfering RNAs (siRNAs), and hairpin RNAs (hpRNAs) has emerged as another technology that could be regarded as more eco-friendly, sustainable, and publicly acceptable than genetic transformation. Inside the plant cell, dsRNAs can undergo several steps of processing, which not only triggers RNAi machinery but may also involve transitive and systemic silencing, as well as epigenetic modifications. Therefore, along with the considerations of proper exogenous applications of dsRNAs, defining their final destination into plant cells is highly relevant. In this review, we highlighted the significance of several factors that affect dsRNA-induced gene silencing, the fate of exogenous dsRNAs in the plant cell, and the challenges surrounding production technologies, cost-effectiveness, and dsRNAs stability under open-field conditions. This review also provided insights into the potential applications of exogenous dsRNAs in plant protection and crop improvement.

Schlagwörter:

agricultural trails; apoplastic movement; application method; delivery technique; exogenous dsRNAs; plant RNAi; symplastic movement

Kategorien:

1.6 not applicable; 2.6 function; 3.3 not applicable; 4.3 not applicable; 5.2 exogenous; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Das, Sumistha; Debnath, Nitai; Cui, Yingjun; Unrine, Jason; Palli, Subba Reddy (2015):

Chitosan, Carbon Quantum Dot, and Silica Nanoparticle Mediated dsRNA Delivery for Gene Silencing in Aedes aegypti: A Comparative Analysis.

In: *ACS applied materials & interfaces* 7 (35), S. 19530–19535. DOI: 10.1021/acsami.5b05232.

Abstract:

In spite of devastating impact of mosquito borne pathogens on humans, widespread resistance to chemical insecticides and environmental concerns from residual toxicity limit mosquito control strategies. We tested three nanoparticles, chitosan, carbon quantum dot (CQD), and silica complexed with dsRNA, to target two mosquito genes (SNF7 and SRC) for controlling Aedes aegypti larvae. Relative mRNA levels were quantified using qRT-PCR to evaluate knockdown efficiency in nanoparticle-dsRNA treated larvae. The knockdown efficiency of target genes correlated with dsRNA mediated larval mortality. Among the three nanoparticles tested, CQD was the most efficient carrier for dsRNA retention, delivery, and thereby causing gene silencing and mortality in Ae. aegypti.

Schlagwörter:

Aedes/genetics/growth & development/metabolism; Animals; Carbon/chemistry; Chitosan/chemistry; Endosomal Sorting Complexes Required for Transport/antagonists & inhibitors/genetics/metabolism; gene silencing; Insect Proteins/antagonists & inhibitors/genetics/metabolism; Larva/genetics/metabolism; Nanoparticles/chemistry/ultrastructure; Quantum Dots/chemistry/ultrastructure; Real-Time Polymerase Chain Reaction; RNA, Double-Stranded/chemistry/metabolism; RNA, Long Noncoding/antagonists & inhibitors/genetics/metabolism; RNA, Messenger/metabolism; Silicon Dioxide/chemistry

Kategorien:

1.6 not applicable; 2.2.24 Aedes aegypti (mosquito); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2.1 mRNA degradation; 5.2 exogenous; 5.2.4 carrier; 5.2.4.1 nanoparticle-mediated dsRNA; 6.1.7 cell differentiation; 6.1.8 cell adhesion; 6.1.9 cell motility; 6.1.15 intracellular transport; 6.1.17 cell proliferation, mitosis; 7.1 Concept development

Zeitschriftenaufsatz

Dietz-Pfeilstetter, Antje; Mendelsohn, Mike; Gathmann, Achim; Klinkenbuß, Dominik (2021):

Considerations and Regulatory Approaches in the USA and in the EU for dsRNA-Based Externally Applied Pesticides for Plant Protection.

In: *Frontiers in Plant Science* 12, S. 682387. DOI: 10.3389/fpls.2021.682387.

Abstract:

Increasing pest and pathogen challenges as well as having fewer conventional pesticides to employ require innovative and sustainable solutions for plant protection. One group of pesticides that is in the pipeline and is expected to be subject to regulation and risk assessment procedures in the near future, is based on the natural gene silencing mechanism RNA interference (RNAi). These dsRNA-based products can be highly specific for a target organism due to the sequence-specific interaction between effective small interfering RNAs (siRNAs) and a complementary target RNA. General regulatory frameworks for pesticide authorization in the U.S. and in the EU are presented. In

addition, production and application procedures and specific characteristics of dsRNA-based pesticides relevant for risk assessment and regulation are considered.

Schlagwörter:

dsRNA; pesticide; pesticide regulation; plant protection; SIGS (spray-induced gene silencing)

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Dong, Yong-Cheng; Wang, Zhi-Jian; Chen, Zhen-Zhong; Clarke, Anthony R.; Niu, Chang-Ying (2016):

Bactrocera dorsalis male sterilization by targeted RNA interference of spermatogenesis: empowering sterile insect technique programs.

In: *Scientific reports* 6, S. 35750. DOI: 10.1038/srep35750.

Abstract:

RNA interference (RNAi) is a genetic technique which has novel application for sustainable pest control. The Sterile Insect Technique (SIT) uses releases of mass-produced, sterile male insects to out-compete wild males for mates to reduce pest populations. RNAi sterilization of SIT males would have several advantages over radiation sterilization, but to achieve this appropriate target genes must first be identified and then targeted with interference technology. With this goal, eight spermatogenesis related candidate genes were cloned and tested for potential activity in Bactrocera dorsalis. The knockdown of candidate genes by oral delivery of dsRNAs did not influence the mating of male flies, but significantly affected the daily average number of eggs laid by females, and reduced egg hatching rate by 16-60%. RNAi negatively affected spermatozoa quantitatively and qualitatively. Following the mating of lola-/topi-/rac-/rho-/upd-/magu-silenced males, we recorded a significant decrease in number and length of spermatozoa in female spermatheca compared to gfp-silenced control group. In a greenhouse trial, the number of damaged oranges and B. dorsalis larvae were significantly reduced in a dsrho-treated group compared with the dsgfp group. This study provides strong evidence for the use RNAi in pest management, especially for the improvement of SIT against B. dorsalis and other species.

Schlagwörter:

Animals; Cloning, Molecular; Female; gene expression; Genes, Insect; Male; Pest Control, Biological/ methods; RNA Interference; Sperm Count; Spermatogenesis/genetics; Tephritidae/genetics/physiology

Kategorien:

1.3.6 Orange; 2.2.15 Bactrocera dorsalis (Oriental fruit fly); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2.1 mRNA degradation; 5.2 exogenous; 6.4.5 spermatogenesis; 7.2 Concept demonstration

Zeitschriftenaufsatz

Fu, Kai-Yun; Meng, Qing-Wei; Lü, Feng-Gong; Guo, Wen-Chao; Ahmat, Tursun; Li, Guo-Qing (2015):

The basic helix–loop–helix transcription factors in the Colorado potato beetle Leptinotarsa decemlineata.

In: *Journal of Asia-Pacific Entomology* 18 (2), S. 197–203. DOI: 10.1016/j.aspen.2015.01.007.

Abstract:

The basic helix-loop-helix (bHLH) transcription factors possess crucial functions in cell proliferation, determination, differentiation, cell cycle maintenance and homeostasis or stress response pathways. Since a few juvenile hormone analogues such as hydroprene, methoprene and pyriproxyfen targeting a bHLH member Met are already registered for pest management, there is a potential to develop more insecticides targeting various bHLHs. The identification of bHLH members is the first step. Based on the transcriptome and the genome of the Colorado potato beetle Leptinotarsa decemlineata, the most important pest in potato, 49 bHLH members were identified. All LdbHLH members were defined by their names and families according to various phylogenetic analyses with bHLH homologues of Drosophila melanogaster, Apis mellifera, Bombyx mod and Tribolium castaneum. Among these LdbHLHs, 17, 10, 10, 1, 10 and 1 members belonged to A, B, C, D, E and F high-order groups, respectively. The results would provide useful background information for future studies on functions of bHLH proteins in the regulation of L. decemlineata development. Moreover, the bHLHs could serve as potential insecticide targets. These proteins could be used in small molecule screen, or in the development of RNAi-based pest management methods. (C) 2015 Korean Society of Applied Entomology, Taiwan Entomological Society and Malaysian Plant Protection Society. Published by Elsevier B.V.

Schlagwörter:

bHLH; genome; identification; Leptinotarsa decemlineata

Kategorien:

1.6 not applicable; 2.2.19 Leptinotarsa decemlineata (Colorado potato beetle); 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.1.2 stress response pathways; 6.1.7 cell differentiation; 6.1.14 cell cycle maintenance; 6.1.17 cell proliferation, mitosis; 7.1 Concept development

Zeitschriftenaufsatz

Fu, Shu; Liu, Zhaoxia; Chen, Jinzhi; Sun, Gengxiao; Jiang, Yingxia; Li, Miaowen et al. (2020):

Silencing arginine kinase/integrin β1 subunit by transgenic plant expressing dsRNA inhibits the development and survival of Plutella xylostella.

In: *Pest management science* 76 (5), S. 1761–1771. DOI: 10.1002/ps.5701.

Abstract:

BACKGROUND

Plutella xylostella is a devastating agricultural insect pest of cruciferous plants, including crops. Plant-mediated RNA interference (RNAi) is currently being developed for plant protection. In this study, we investigated the response of P. xylostella exposed to transgenic Arabidopsis thaliana plants that expressed double-stranded RNA (dsRNA) targeting P. xylostella genes of arginine kinase (PxAK) and integrin β1 subunit (Pxβ).

RESULTS

Transgenic plants producing dsRNAs of the 384-bp fragment of PxAK (dsAK plants), the 497-bp fragment of Pxβ (dsβ plants), and the 881 bp of the combination of both genes (dsAK-β plants) were generated and verified. Insect bioassay with these transgenic plants showed that the development of P. xylostella was affected, causing longer developmental time, and lower pupal weight and pupation rate. P. xylostella mortality rates were 25.0% when exposed to dsAK plants, 22.5% with dsβ plants, and 30.0% with dsAK-β plants, which were all higher than 7.5% for the wild-type plant. PxAK and Pxβ in P. xylostella were suppressed by 26.6-79.7% at the transcription level by the transgenic plants.

CONCLUSION

These results suggest that plant-mediated RNAi targeting single gene or both PxAK and Pxβ may have the potential to control P. xylostella. © 2019 Society of Chemical Industry.

Schlagwörter:

Animals; Arginine Kinase; gene silencing; Integrin beta1; Moths; Plants, Genetically Modified; RNA Interference; RNA, Double-Stranded/genetics

Kategorien:

1.5.3 Arabidopsis thaliana; 2.2.11 Plutella xylostella (Diamondback moth); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.1 endogenous; 5.1.1 stable transgene; 6.1.12 cytosceleton; 6.3 energy metabolism; 7.2 Concept demonstration

Zeitschriftenaufsatz

Gaffar, Fatima Yousif; Imani, Jafargholi; Karlovsky, Petr; Koch, Aline; Kogel, Karl-Heinz (2019):

Different Components of the RNA Interference Machinery Are Required for Conidiation, Ascosporogenesis, Virulence, Deoxynivalenol Production, and Fungal Inhibition by Exogenous Double-Stranded RNA in the Head Blight Pathogen Fusarium graminearum.

In: *Frontiers in Microbiology* 10, S. 1662. DOI: 10.3389/fmicb.2019.01662.

Abstract:

In filamentous fungi, gene silencing through RNA interference (RNAi) shapes many biological processes, including pathogenicity. We explored the requirement of key components of fungal RNAi machineries, including DICER-like 1 and 2 (FgDCL1, FgDCL2), ARGONAUTE 1 and 2 (FgAGO1, FgAGO2), AGO-interacting protein FgQIP (QDE2-interacting protein), RecQ helicase (FgQDE3), and four RNA-dependent RNA polymerases (FgRdRP1, FgRdRP2, FgRdRP3, FgRdRP4), in the ascomycete mycotoxin-

producing fungal pathogen Fusarium graminearum (Fg) for sexual and asexual multiplication, pathogenicity, and its sensitivity to double-stranded (ds)RNA. We corroborate and extend earlier findings that conidiation, ascosporogenesis, and Fusarium head blight (FHB) symptom development require an operable RNAi machinery. The involvement of RNAi in conidiation is dependent on environmental conditions as it is detectable only under low light (<2 µmol m-2 s-1). Although both DCLs and AGOs partially share their functions, the sexual ascosporogenesis is mediated primarily by FgDCL1 and FgAGO2, while FgDCL2 and FgAGO1 contribute to asexual conidia formation and germination. FgDCL1 and FgAGO2 also account for pathogenesis as their knockout (KO) results in reduced FHB development. Apart from KO mutants Δdcl2 and Δago1, mutants Δrdrp2, Δrdrp3, Δrdrp4, Δqde3, and Δqip are strongly compromised for conidiation, while KO mutations in all RdPRs, QDE3, and QIP strongly affect ascosporogenesis. Analysis of trichothecenes mycotoxins in wheat kernels showed that the relative amount of deoxynivalenol (DON), calculated as [DON] per amount of fungal genomic DNA was reduced in all spikes infected with RNAi mutants, suggesting the possibility that the fungal RNAi pathways affect Fg's DON production. Moreover, silencing of fungal genes by exogenous target gene-specific double-stranded RNA (dsRNA) (spray-induced gene silencing, SIGS) is dependent on DCLs, AGOs, and QIP, but not on QDE3. Together these data show that in F. graminearum, different key components of the RNAi machinery are crucial in different steps of fungal development and pathogenicity.

Schlagwörter:

argonaute; dsRNA; Fusarium graminearium; SIGS (spray-induced gene silencing); small RNA; wheat (Triticum aestivum)

Kategorien:

1.6 not applicable; 2.1.1 Fusarium graminearum; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Gaffar, Fatima Yousif; Koch, Aline (2019):

Catch Me If You Can! RNA Silencing-Based Improvement of Antiviral Plant Immunity.

In: *Viruses* 11 (7). DOI: 10.3390/v11070673.

Abstract:

Viruses are obligate parasites which cause a range of severe plant diseases that affect farm productivity around the world, resulting in immense annual losses of yield. Therefore, control of viral pathogens continues to be an agronomic and scientific challenge requiring innovative and ground-breaking strategies to meet the demands of a growing world population. Over the last decade, RNA silencing has been employed to develop plants with an improved resistance to biotic stresses based on their function to provide protection from invasion by foreign nucleic acids, such as viruses. This natural phenomenon can be exploited to control agronomically relevant plant diseases. Recent evidence argues that this biotechnological method, called host-induced gene silencing, is effective against sucking insects, nematodes, and pathogenic fungi, as well as bacteria and viruses on their plant hosts. Here, we review recent studies which reveal the enormous potential that RNA-silencing

strategies hold for providing an environmentally friendly mechanism to protect crop plants from viral diseases.

Schlagwörter:

Disease Resistance/genetics/immunology; gene silencing; Host-Pathogen Interactions/genetics/immunology; Plant Diseases/genetics/virology; Plant Physiological Phenomena; Plant Viruses; Plants/genetics/immunology/virology; RNA Interference

Kategorien:

1.6 not applicable; 2.4 viruses; 3.3 not applicable; 4.3 not applicable; 5.1 endogenous; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Ghosh, Snigdha; Patra, Snehanjana; Ray, Sarmistha (2023):

A Combinatorial Nanobased Spray-Induced Gene Silencing Technique for Crop Protection and Improvement.

In: *ACS omega* 8 (25), S. 22345–22351. DOI: 10.1021/acsomega.3c01968.

Abstract:

Recent research reports have shown that plant pests and pathogens have depleted the crop yield widely, which has led to an increased dependence on commercial pesticides and fungicides. Increased usage of these pesticides has also shown adverse effects on the environment, therefore many techniques have been implemented for solving the issue, some of which include using nanobioconjugates, RNA(i), which put into use double-stranded RNAs to inhibit gene expression. A more innovative and eco-friendly strategy includes spray induced gene silencing, which is being increasingly implemented. This review delves into the eco-friendly approach of spray induced gene silencing (SIGS) in combination with nanobioconjugates, which have been used concerning various plant hosts and their pathogens to provide improved protection. Furthermore, nanotechnological advancements have been understood by addressing the scientific gaps to provide a rationale for the development of updated techniques in crop protection.

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.2.2 spray (SIGS); 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Girard, Ian J.; Mcloughlin, Austein G.; Kievit, Teresa R. de; Fernando, Dilantha W. G.; Belmonte, Mark F. (2016):

Integrating Large-Scale Data and RNA Technology to Protect Crops from Fungal Pathogens.

In: *Frontiers in Plant Science* 7, S. 631. DOI: 10.3389/fpls.2016.00631.

Abstract:

With a rapidly growing human population it is expected that plant science researchers and the agricultural community will need to increase food productivity using less arable land. This challenge is complicated by fungal pathogens and diseases, many of which can severely impact crop yield. Current measures to control fungal pathogens are either ineffective or have adverse effects on the agricultural enterprise. Thus, developing new strategies through research innovation to protect plants from pathogenic fungi is necessary to overcome these hurdles. RNA sequencing technologies are increasing our understanding of the underlying genes and gene regulatory networks mediating disease outcomes. The application of invigorating next generation sequencing strategies to study plant-pathogen interactions has and will provide unprecedented insight into the complex patterns of gene activity responsible for crop protection. However, questions remain about how biological processes in both the pathogen and the host are specified in space directly at the site of infection and over the infection period. The integration of cutting edge molecular and computational tools will provide plant scientists with the arsenal required to identify genes and molecules that play a role in plant protection. Large scale RNA sequence data can then be used to protect plants by targeting genes essential for pathogen viability in the production of stably transformed lines expressing RNA interference molecules, or through foliar applications of double stranded RNA.

Kategorien:

1.6 not applicable; 2.1 fungi; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Goodfellow, Simon; Zhang, Daai; Wang, Ming-Bo; Zhang, Ren (2019):

Bacterium-Mediated RNA Interference: Potential Application in Plant Protection.

In: *Plants (Basel, Switzerland)* 8 (12). DOI: 10.3390/plants8120572.

Abstract:

RNAi has emerged as a promising tool for targeting agricultural pests and pathogens and could provide an environmentally friendly alternative to traditional means of control. However, the deployment of this technology is still limited by a lack of suitable exogenous- or externally applied delivery mechanisms. Numerous means of overcoming this limitation are being explored. One such method, bacterium-mediated RNA interference, or bmRNAi, has been explored in other systems and shows great potential for application to agriculture. Here, we review the current state of bmRNAi, examine the technical limitations and possible improvements, and discuss its potential applications in crop protection.

Schlagwörter:

bacterial-mediated RNAi (bm-RNAi); disease control; plant protection; RNAi (RNA interference)

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.2 exogenous; 5.2.3 bmRNAi; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Grover, Sajjan; Jindal, Vikas; Banta, Geetika; Taning, Clauvis Nji Tizi; Smagghe, Guy; Christiaens, Olivier (2019):

Potential of RNA interference in the study and management of the whitefly, Bemisia tabaci.

In: *Archives of insect biochemistry and physiology* 100 (2), e21522. DOI: 10.1002/arch.21522.

Abstract:

Whiteflies cause considerable losses to crops, directly by feeding, and indirectly by transmission of viruses. The current control methods consist of a combination of different control tactics, mainly still relying on unsafe and non-ecofriendly chemical control. RNA interference (RNAi) is a post-transcriptional gene-silencing strategy in which double-stranded RNA (dsRNA), corresponding specifically to a target gene, is introduced in a target organism. Research on RNAi in the previous decade has shown its success as a potential insect control strategy, which can be highly species-specific and environment friendly. In whiteflies, the success of dsRNA delivery through the oral route opened possibilities for its management through plant-mediated RNAi. To date, several genes have been targeted in whiteflies through RNAi and these assays demonstrated its potential to manage whiteflies at lab level. However, further research and investments are needed to move toward an application at field level. In this review, for the first time, we collected the literature on genes targeted for silencing via RNAi in whiteflies and discuss the potential of RNAi in whitefly pest control. We also discuss likely delivery methods, including transgenic in planta delivery and symbiont-mediated delivery, and its potential for studying and interfering with insecticide resistance mechanisms and virus transmission by whiteflies.

Schlagwörter:

Animals; Hemiptera/genetics/physiology; insect control; RNA Interference

Kategorien:

1.6 not applicable; 2.2.10 Bemisia tabaci (White fly); 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Gu, Kai-Xin; Song, Xiu-Shi; Xiao, Xue-Mei; Duan, Xiao-Xin; Wang, Jian-Xin; Duan, Ya-Bing et al. (2019):

A β2-tubulin dsRNA derived from Fusarium asiaticum confers plant resistance to multiple phytopathogens and reduces fungicide resistance.

In: *Pesticide biochemistry and physiology* 153, S. 36–46. DOI: 10.1016/j.pestbp.2018.10.005.

Abstract:

Crops are attacked by a large number of pathogens which are responsible for an approximately 30% loss in global crop production at pre- and post-harvest levels. In light of the continuing emergence of fungicide resistance, the needs for new agricultural drugs turn out to be much more critical. Here we demonstrated a Faβ2Tub-3 dsRNA derived from Fusarium asiaticum had broad-spectrum antifungal activity against Fusarium spp., Botrytis cinerea, Magnaporthe oryzae and Colletotrichum truncatum, with an additional function of reducing the dosage of carbendazim (MBC) fungicide. RNAi molecules derived from different regions of β2-tubulin gene had different effects on mycelial growth, asexual reproduction and virulence. Faβ2Tub-3 (one of β2-tubulin segments) exhibited a strong silencing efficacy both on β1-tubulin and β2-tubulin genes in F. asiaticum. Faβ2Tub-3 sequence was found to be highly conserved among Fusarium spp., Botrytis cinerea, Magnaporthe oryzae and Colletotrichum truncatum. The Faβ2Tub-3 dsRNA demonstrated a broad-spectrum antifungal activity against these fungi in vitro and on living plant. More importantly, Faβ2Tub-3 dsRNA increased the fungal sensitivity to MBC, while MBC increased the duration of Faβ2Tub-3 dsRNA. Our findings suggest a new anti-fungal agent (Faβ2Tub-3 dsRNA) for plant protection against diverse pathogens and for fungicide reduction.

Schlagwörter:

disease resistance; Drug Resistance, Fungal/genetics; Fungal Proteins/genetics; Fungicides, Industrial/toxicity; Fusarium/genetics/pathogenicity; RNA, Double-Stranded/genetics; RNA, Fungal/genetics; Triticum/microbiology; Tubulin/genetics

Kategorien:

1.6 not applicable; 2.1.2 Botrytis cinerea; 2.1.7 Fusarium spp.; 2.1.8 Magnaporthe oryzae; 2.1.9 Colletotrichum truncatum; 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.3 not applicable; 6.1.9 cell motility; 6.1.15 intracellular transport; 6.1.17 cell proliferation, mitosis; 7.1 Concept development

Zeitschriftenaufsatz

Guo, Huan; Liu, Xuan-Zheng; Long, Gui-Jun; Gong, Lang-Lang; Zhang, Meng-Qi; Ma, Yun-Feng et al. (2022):

Functional characterization of developmentally critical genes in the white-backed planthopper: Efficacy of nanoparticle-based dsRNA sprays for pest control.

In: *Pest management science*. DOI: 10.1002/ps.7271.

Abstract:

BACKGROUND

Epidermal growth factor receptor (EGFR), zinc finger homeodomain-2 (zfh-2), Abdominal-A (Abd-A), and Abdominal-B (Abd-B) regulate the growth and development of the insect abdomen. However, their potential roles in pest control have not been fully assessed. The development of insecticide resistance to multiple chemistries in the white-backed planthopper (WBPH), a major pest of rice, has prompted interest in novel pest control approaches that are ecologically friendly. Although pest management approaches based on double-stranded RNA (dsRNA)-mediated RNA interference (RNAi) have potential, their susceptibility to degradation limits large-scale field applications. These limitations, however, can be overcome with nanoparticle-dsRNA complexes that have greater environmental stability and improved cellular uptake.

RESULTS

In this study, at 5 days post-injection, transcripts for the four gene targets were reduced relative to controls and all of the experimental groups exhibited significant phenotypic defects and increased mortality. To evaluate the potential of these gene targets for field applications, a nanocarrier-dsRNA spray delivery system was assessed for RNAi efficacy. At 11 days post-spray, significant phenotypic defects and increased mortality were observed in all experimental groups.

CONCLUSION

Taken together, the results confirm the suitability of the target genes (SfEGFR, Sfzfh-2, SfAbd-A, and SfAbd-B) for pest management and demonstrate the efficacy of the nanocarrier spray system for inducing RNAi-mediated knockdown. As such, the study lays the foundation for the further development and optimization of this technology for large-scale field applications. © 2022 Society of Chemical Industry.

Schlagwörter:

abdominal-A; abdominal-B; growth factor receptor; nanoparticle-wrapped; RNAi; SIGS (spray-induced gene silencing); white-backed planthopper; zinc finger homeodomain-2

Kategorien:

1.1.4 Oryza sativa (rice); 2.2.23 Sogatella furcifera (White-backed planthopper); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2 exogenous; 5.2.4.1 nanoparticle-mediated dsRNA; 6.2.1 abdomen growth and development; 7.1 Concept development

Zeitschriftenaufsatz

GUPTA, N. C.; RAO, MAHESH; SHARMA, PANKAJ (2018):

MOLECULAR ASPECTS OF SCLEROTINIA STEM ROT DISEASE MANAGEMENT IN OILSEED CROPS.

In: *BIOP* 10 (4), S. 1166. DOI: 10.9735/0975-5276.10.4.1166-1170.

Abstract:

The pathogens aggression with endless evolutionary pressure refines their molecular strategies to achieve the successful path ogenesis. Sclerotinia

sclerotiorum (Lib) de Bary, a necrotrophic phytopathogen is ubiquitously distributed worldwide and affecting the large number of host species. Several control measures

like fungicides application, cultural practices, crop rotation are the usual practices available to the farmers are in use. But, despite its success, these processes are quite

expensive and indistinctness of fungicides doses and time of application are the major hurdle in their routine use. Although, partial resistance/tolerance has already

been reported in B. napus and B. carinata but not a single source in B. juncea, which hinders the resistance breeding program. However, the recent advancement in

biotechnological interventions are observed more promising in developing the alternatives like fungal growth inhibition, defense response activation, detoxification of the

virulence factors, and RNAi or HIGS for engineered resistance to the Sclerotinia stem rot disease.

Schlagwörter:

anti-microbial peptides; oilseed; oxalic acid; Sclerotinia; stem rot

Kategorien:

1.2.3 oilseed crops; 2.1.3 Sclerotinia sclerotiorum; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Haj Darwich, Choukri M.; Chrzanowski, Marcin M.; Bernatowicz, Piotr P.; Polanska, Marta A.; Joachimiak, Ewa; Bebas, Piotr (2022):

Molecular Oscillator Affects Susceptibility of Caterpillars to Insecticides: Studies on the Egyptian Cotton Leaf Worm-Spodoptera littoralis (Lepidoptera: Noctuidae).

In: *Insects* 13 (5). DOI: 10.3390/insects13050488.

Abstract:

The molecular oscillator is the core of the biological clock and is formed by genes and proteins whose cyclic expression is regulated in the transcriptional-translational feedback loops (TTFLs). Proteins of the TTFLs are regulators of both their own and executive genes involved in the control of many processes in insects (e.g., rhythmic metabolism of xenobiotics, including insecticides). We disrupted the clock operation in S. littoralis larvae by injecting the dsRNA of clock genes into their body cavity and culturing the larvae under continuous light. As a result, the daily susceptibility of larvae to insecticides was abolished and the susceptibility itself increased (in most cases). In the fat body, midgut, and Malpighian tubules (the main organs metabolizing xenobiotics) of the larvae treated with injected-dsRNA, the daily activity profiles of enzymes involved in detoxification-cytochrome P450 monooxygenases, Glutathione-S-transferase, and esterase-have changed significantly. The presented results prove the role of the molecular oscillator in the regulation of larvae responses to insecticides and provide grounds for rational use of these compounds (at suitable times of the day), and may indicate clock genes as potential targets of molecular manipulation to produce plant protection compounds based on the RNAi method.

Schlagwörter:

circadian clock; Lepidoptera/genetics; molecular oscillator; Spodoptera littoralis; susceptibility to insecticides

Kategorien:

1.6 not applicable; 2.2.27 Spodoptera littoralis (Egyptian Cotton Leaf Worm); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2.9 microinjection; 6.1.1 biological clock; 7.1 Concept development

Zeitschriftenaufsatz

Hajeri, Subhas; Killiny, Nabil; El-Mohtar, Choaa; Dawson, William O.; Gowda, Siddarame (2014):

Citrus tristeza virus-based RNAi in citrus plants induces gene silencing in Diaphorina citri, a phloem-sap sucking insect vector of citrus greening disease (Huanglongbing).

In: *Journal of biotechnology* 176, S. 42–49. DOI: 10.1016/j.jbiotec.2014.02.010.

Abstract:

A transient expression vector based on Citrus tristeza virus (CTV) is unusually stable. Because of its stability it is being considered for use in the field to control Huanglongbing (HLB), which is caused by Candidatus Liberibacter asiaticus (CLas) and vectored by Asian citrus psyllid, Diaphorina citri. In the absence of effective control strategies for CLas, emphasis has been on control of D. citri. Coincident cohabitation in phloem tissue by CLas, D. citri and CTV was exploited to develop a novel method to mitigate HLB through RNA interference (RNAi). Since CTV has three RNA silencing suppressors, it was not known if CTV-based vector could induce RNAi in citrus. Yet, expression of sequences targeting citrus phytoene desaturase gene by CTV-RNAi resulted in photo-bleaching phenotype. CTV-RNAi vector, engineered with truncated abnormal wing disc (Awd) gene of D. citri, induced altered Awd expression when silencing triggers ingested by feeding D. citri nymphs. Decreased Awd in nymphs resulted in malformed-wing phenotype in adults and increased adult mortality. This impaired ability of D. citri to fly would potentially limit the successful vectoring of CLas bacteria between citrus trees

in the grove. CTV-RNAi vector would be relevant for fast-track screening of candidate sequences for RNAi-mediated pest control.

Schlagwörter:

Animals; Citrus/genetics/microbiology; Closterovirus/classification/genetics; gene silencing; Genes, Insect; Hemiptera/genetics/physiology; Nymph/genetics; Oxidoreductases/genetics; Phloem/microbiology; Plant Diseases/microbiology; Plant Proteins/genetics; Rhizobiaceae/physiology; RNA Interference; RNA, Viral/genetics

Kategorien:

1.3.5 Citrus sp.; 2.2.22 Diaphorina citri; 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2.1 VIGS; 6.1.10 maintenance of epithelial integrity; 7.2 Concept demonstration

Zeitschriftenaufsatz

Hamby, Rachael; Wang, Ming; Qiao, Lulu; Jin, Hailing (2020):

Synthesizing Fluorescently Labeled dsRNAs and sRNAs to Visualize Fungal RNA Uptake.

In: *Methods in molecular biology (Clifton, N.J.)* 2166, S. 215–225. DOI: 10.1007/978-1-0716-0712-1_12.

Abstract:

Fungal pathogens are responsible for severe crop losses worldwide. Defending crops against fungal disease is critical for global food security; however, most current disease management approaches rely on chemical fungicides that can leave dangerous residues in the environment. RNA interference (RNAi) is an important process through which RNA molecules target and silence complementary genes, regulating gene expression during both transcription and translation. Recently, it has been discovered that some species of fungi can efficiently take up RNAs originating from their host plant and the environment. If these RNAs are complementary to fungal genes, this can lead to the targeting and silencing of fungal genes, termed "cross-kingdom RNAi," if the RNA originated from a plant host, or "environmental RNAi," if the RNA originated from the environment. These discoveries have inspired the development of spray-induced gene silencing (SIGS), an innovative crop protection strategy involving the foliar application of RNAs which target and silence fungal virulence genes for plant protection against fungal pathogens. The effectiveness of SIGS is largely dependent on the ability of fungi to take up environmental RNAs. Here, we describe the protocols used to label and visualize RNAs which are taken up by Botrytis cinerea. This protocol could easily be adapted for use across various fungal species. Determining the efficiency of RNA uptake by a specific fungal species is a critical first step to determining if SIGS approaches could be an effective control strategy for that fungus.

Schlagwörter:

Biological Transport/genetics; Botrytis/genetics; crop protection; cross-kingdom RNAi; dsRNA; environmental RNAi; Fluorescence; Fungi/genetics/pathogenicity; fungicides; gene silencing; Microscopy, Fluorescence/methods; Plant Diseases/genetics/microbiology/therapy; RNA Interference; RNA labeling; RNA uptake; RNA, Double-Stranded/chemical

synthesis/pharmacology/therapeutic use; RNA, Fungal/metabolism; RNA, Plant/chemistry/genetics; RNAi (RNA interference); SIGS (spray-induced gene silencing); Virulence/genetics

Kategorien:

1.6 not applicable; 2.1.2 Botrytis cinerea; 3.3 not applicable; 4.3 not applicable; 5.2.2 spray (SIGS); 6.9 not applicable; 7.1 Concept development

Zeitschriftenaufsatz

He, Bicheng; Chu, Yuan; Yin, Meizhen; Müllen, Klaus; An, Chunju; Shen, Jie (2013):

Fluorescent nanoparticle delivered dsRNA toward genetic control of insect pests.

In: *Advanced materials (Deerfield Beach, Fla.)* 25 (33), S. 4580–4584. DOI: 10.1002/adma.201301201.

Abstract:

A fluorescent cationic core-shell nanoparticle efficiently enters into cells with high transfection efficacy. A FNP/CHT10-dsRNA complex is orally fed to insect pests and knocks down a midgut-specific chitinase gene of the Asian corn borer, which leads to death. This is the first report on the genetic control of insect pests through a non-viral gene delivery system to knock down key developmental gene expression.

Schlagwörter:

Animals; Apoptosis/drug effects; Cell Survival/drug effects; Dose-Response Relationship, Drug; Drosophila/cytology/drug effects/genetics; Fluorescent Dyes/chemistry; Genetic Vectors/chemistry; insect control; Insecticides/pharmacology; Molecular Structure; Nanoparticles/chemistry; RNA, Double-Stranded/chemistry/genetics; Structure-Activity Relationship

Kategorien:

1.6 not applicable; 2.2.25 Ostrinia furnacalis (Asian corn borer); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2 exogenous; 5.2.4.1 nanoparticle-mediated dsRNA; 6.2.4 molting; 6.2.5.1 metamorphosis; 7.1 Concept development

Zeitschriftenaufsatz

He, Li; Huang, Yanna; Tang, Xueming (2022):

RNAi-based pest control: Production, application and the fate of dsRNA.

In: *Frontiers in Bioengineering and Biotechnology* 10, S. 1080576. DOI: 10.3389/fbioe.2022.1080576.

Abstract:

The limitations of conventional pesticides have raised the demand for innovative and sustainable solutions for plant protection. RNA Interference (RNAi) triggered by dsRNA has evolved as a promising strategy to control insects in a species-specific manner. In this context, we review the

methods for mass production of dsRNA, the approaches of exogenous application of dsRNA in the field, and the fate of dsRNA after application. Additionally, we describe the opportunities and challenges of using nanoparticles as dsRNA carriers to control insects. Furthermore, we provide future directions to improve pest management efficiency by utilizing the synergistic effects of multiple target genes. Meanwhile, the establishment of a standardized framework for assessment and regulatory consensus is critical to the commercialization of RNA pesticides.

Schlagwörter:

mass production; RNA biopesticides

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.2 exogenous; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

He, Wanwan; Xu, Wenbo; Fu, Kaiyun; Guo, Wenchao; Kim, Dae Sung; Zhang, Jiang (2022):

Positional effects of double-stranded RNAs targeting β-Actin gene affect RNA interference efficiency in Colorado potato beetle.

In: *Pesticide biochemistry and physiology* 184, S. 105121.

Abstract:

Pesticide resistance in pests drives the development of RNA interference (RNAi)-based technology as a novel approach for pest control. To investigate the effects of the positional dependency of double-stranded RNAs (dsRNAs), we newly designed four different 200 bp dsRNAs targeting Colorado potato beetle (CPB) β-Actin gene, termed as dsACT200-1 to dsACT200-4, to compare their insecticidal activity to CPB larvae together with our previously used 200 bp and 700 bp dsRNAs (dsACT200 and dsACT700), respectively (He et al., 2020a). Each of dsRNAs harbors different numbers of expected siRNAs predicted by sequence-based prediction platform, dsACT200 and dsACT200-2 have a relatively higher number of siRNA than other 200 bps dsRNAs. When CPB larvae were fed with in vitro synthesized dsRNA-painted potato leaves, all the tested dsRNAs showed significant effects to protect against CPB larvae. Combined with the survival rate of CPB larvae, β-Actin gene expression level and the surviving CPB larvae weight, various positional dsRNAs from the same allele showed different plant protection activity against CPB larvae and partially correlated with the predicted siRNA numbers and distribution on the target sequence. This study suggests the specific allelic locus for rational dsRNA design triggering RNAi efficiency for target gene silencing is an essential factor in enhancing the insecticidal activity.

Schlagwörter:

Actins/genetics/metabolism/pharmacology; Animals; Coleoptera; Insecticides/pharmacology; RNA Interference; RNA, Double-Stranded/genetics/pharmacology; RNA, Small Interfering/genetics/metabolism; Solanum tuberosum/genetics/metabolism

Kategorien:

1.4.5 Solanum tuberosum (potato); 2.2.19 Leptinotarsa decemlineata (Colorado potato beetle); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2.2 spray (SIGS); 6.1.12 cytosceleton; 7.2 Concept demonstration

Zeitschriftenaufsatz

He, Wanwan; Xu, Wenbo; Xu, Letian; Fu, Kaiyun; Guo, Wenchao; Bock, Ralph; Zhang, Jiang (2020):

Length-dependent accumulation of double-stranded RNAs in plastids affects RNA interference efficiency in the Colorado potato beetle.

In: *Journal of experimental botany* 71 (9), S. 2670–2677. DOI: 10.1093/jxb/eraa001.

Abstract:

Transplastomic potato plants expressing double-stranded RNA (dsRNA) targeted against essential genes of the Colorado potato beetle (CPB) can be lethal to larvae by triggering an RNA interference (RNAi) response. High accumulation levels of dsRNAs in plastids are crucial to confer an efficient RNAi response in the insects. However, whether length and sequence of the dsRNA determine the efficacy of RNAi and/or influence the level of dsRNA accumulation in plastids is not known. We compared the RNAi efficacy of different lengths of dsRNA targeted against the CPB β-Actin gene (ACT) by feeding in vitro-synthesized dsRNAs to larvae. We showed that, while the 60 bp dsRNA induced only a relatively low RNAi response in CPB, dsRNAs of 200 bp and longer caused high mortality and similar larval growth retardation. When the dsRNAs were expressed from the plastid (chloroplast) genome of potato plants, we found that their accumulation were negatively correlated with length. The level of dsRNA accumulation was positively associated with the observed mortality, suppression of larval growth, and suppression of target gene expression. Importantly, transplastomic potato plants expressing the 200 bp dsRNA were better protected from CPB than plants expressing the 297 bp dsRNA, the best-performing line in our previous study. Our results suggest that the length of dsRNAs is an important factor that influences their accumulation in plastids and thus determines the strength of the insecticidal RNAi effect. Our findings will aid the design of optimized dsRNA expression constructs for plant protection by plastid-mediated RNAi.

Schlagwörter:

Animals; Coleoptera/genetics; Plastids; RNA Interference; RNA, Double-Stranded/genetics; Solanum tuberosum/genetics

Kategorien:

1.4.5 Solanum tuberosum (potato); 2.2.19 Leptinotarsa decemlineata (Colorado potato beetle); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.1.1 stable transgene; 5.1.1.1 production in chloroplasts; 6.1.12 cytosceleton; 7.2 Concept demonstration

Zeitschriftenaufsatz

Head, Graham P.; Carroll, Matthew W.; Evans, Sean P.; Rule, Dwain M.; Willse, Alan R.; Clark, Thomas L. et al. (2017):

Evaluation of SmartStax and SmartStax PRO maize against western corn rootworm and northern corn rootworm: efficacy and resistance management.

In: *Pest management science* 73 (9), S. 1883–1899. DOI: 10.1002/ps.4554.

Abstract:

BACKGROUND

Cases of western corn rootworm (WCR) field-evolved resistance to Cry3Bb1 and other corn rootworm (CRW) control traits have been reported. Pyramid products expressing multiple CRW traits can delay resistance compared to single trait products. We used field studies to assess the pyramid CRW corn products, SmartStax (expressing Cry3Bb1 and Cry34Ab1/Cry35Ab1) and SmartStax PRO (expressing Cry3Bb1, Cry34Ab1/Cry35Ab1 and DvSnf7), at locations with high WCR densities and possible Cry3Bb1 resistance, and to assess the reduction in adult emergence attributable to DvSnf7 and other traits. Insect resistance models were used to assess durability of SmartStax and SmartStax PRO to WCR resistance.

RESULTS

SmartStax significantly reduced root injury compared to non-CRW-trait controls at all but one location with measurable WCR pressure, while SmartStax PRO significantly reduced root injury at all locations, despite evidence of Cry3Bb1 resistance at some locations. The advantage of SmartStax PRO over SmartStax in reducing root damage was positively correlated with root damage on non-CRW-trait controls. DvSnf7 was estimated to reduce WCR emergence by approximately 80-95%, which modeling indicated will improve durability of Cry3Bb1 and Cry34Ab1/Cry35Ab1 compared to SmartStax.

CONCLUSION

The addition of DvSnf7 in SmartStax PRO can reduce root damage under high WCR densities and prolong Cry3Bb1 and Cry34Ab1/Cry35Ab1 durability. © 2017 Society of Chemical Industry.

Schlagwörter:

Animals; Biological Assay; Coleoptera/physiology; Plant Diseases; Plants, Genetically Modified; Zea mays/genetics/physiology

Kategorien:

1.1.1 Zea mays (maize); 2.2.2 Diabrotica virgifera virgifera (Western corn rootworm); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 5.1 endogenous; 5.1.1 stable transgene; 6.1.15 intracellular transport; 7.2 Concept demonstration

Zeitschriftenaufsatz

Hernández-Soto, Alejandro; Chacón-Cerdas, Randall (2021):

RNAi Crop Protection Advances.

In: *International journal of molecular sciences* 22 (22). DOI: 10.3390/ijms222212148.

Abstract:

RNAi technology is a versatile, effective, safe, and eco-friendly alternative for crop protection. There is plenty of evidence of its use through host-induced gene silencing (HIGS) and emerging evidence that spray-induced gene silencing (SIGS) techniques can work as well to control viruses, bacteria, fungi, insects, and nematodes. For SIGS, its most significant challenge is achieving stability and avoiding premature degradation of RNAi in the environment or during its absorption by the target organism. One alternative is encapsulation in liposomes, virus-like particles, polyplex nanoparticles, and bioclay, which can be obtained through the recombinant production of RNAi in vectors, transgenesis, and micro/nanoencapsulation. The materials must be safe, biodegradable, and stable in multiple chemical environments, favoring the controlled release of RNAi. Most of the current research on encapsulated RNAi focuses primarily on oral delivery to control insects by silencing essential genes. The regulation of RNAi technology focuses on risk assessment using different approaches; however, this technology has positive economic, environmental, and human health implications for its use in agriculture. The emergence of alternatives combining RNAi gene silencing with the induction of resistance in crops by elicitation and metabolic control is expected, as well as multiple silencing and biotechnological optimization of its large-scale production.

Schlagwörter:

crop protection; Crops, Agricultural/genetics/microbiology/parasitology; Humans; Plant Diseases/microbiology/parasitology/prevention & control; RNA Interference; RNA, Small Interfering

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.2.4 carrier; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Hoang, Bao Tram L.; Fletcher, Stephen J.; Brosnan, Christopher A.; Ghodke, Amol B.; Manzie, Narelle; Mitter, Neena (2022):

RNAi as a Foliar Spray: Efficiency and Challenges to Field Applications.

In: *International journal of molecular sciences* 23 (12). DOI: 10.3390/ijms23126639.

Abstract:

RNA interference (RNAi) is a powerful tool that is being increasingly utilized for crop protection against viruses, fungal pathogens, and insect pests. The non-transgenic approach of spray-induced gene silencing (SIGS), which relies on spray application of double-stranded RNA (dsRNA) to induce RNAi, has come to prominence due to its safety and environmental benefits in addition to its wide host range and high target specificity. However, along with promising results in recent studies, several factors limiting SIGS RNAi efficiency have been recognized in insects and plants. While sprayed dsRNA on the plant surface can produce a robust RNAi response in some chewing insects, plant uptake and systemic movement of dsRNA is required for delivery to many other target organisms. For example, pests such as sucking insects require the presence of dsRNA in vascular tissues, while many fungal pathogens are predominately located in internal plant tissues. Investigating the mechanisms by which sprayed dsRNA enters and moves through plant tissues and understanding the barriers that may hinder this process are essential for developing efficient ways to deliver dsRNA into plant systems. In this review, we assess current knowledge of the plant foliar and cellular uptake of dsRNA molecules. We will also identify major barriers to uptake, including leaf morphological features as well as environmental factors, and address methods to overcome these barriers.

Schlagwörter:

Animals; crop protection; gene silencing; Insecta/genetics; RNA Interference; RNA, Double-Stranded/genetics

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.2.2 spray (SIGS); 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Höfle, L.; Biedenkopf, D.; Werner, B. T.; Shrestha, A.; Jelonek, L.; Koch, A. (2020):

Study on the efficiency of dsRNAs with increasing length in RNA-based silencing of the Fusarium CYP51 genes.

In: *RNA biology* 17 (4), S. 463–473. DOI: 10.1080/15476286.2019.1700033.

Abstract:

Previously, we have demonstrated that transgenic Arabidopsis and barley plants, expressing a 791 nucleotide (nt) dsRNA (CYP3RNA) that targets all three CYP51 genes (FgCYP51A, FgCYP51B, FgCYP51C) in Fusarium graminearum (Fg), inhibited fungal infection via a process designated as host-induced gene silencing (HIGS). More recently, we have shown that spray applications of CYP3RNA also protect barley from fungal infection via a process termed spray-induced gene silencing (SIGS). Thus, RNAi technology may have the potential to revolutionize plant protection in agriculture. Therefore, successful field application will require optimization of RNAi design necessary to maximize the efficacy of the RNA silencing construct for making RNAi-based strategies a realistic and sustainable approach in agriculture. Previous studies indicate that silencing is correlated with the

number of siRNAs generated from a dsRNA precursor. To prove the hypothesis that silencing efficiency is correlated with the number of siRNAs processed out of the dsRNA precursor, we tested in a HIGS and SIGS approach dsRNA precursors of increasing length ranging from 400 nt to 1500 nt to assess gene silencing efficiency of individual FgCYP51 genes. Concerning HIGS-mediated disease control, we found that there is no significant correlation between the length of the dsRNA precursor and the reduction of Fg infection on CYP51-dsRNA-expressing Arabidopsis plants. Importantly and in clear contrast to HIGS, we measured a decrease in SIGS-mediated Fg disease resistance that significantly correlates with the length of the dsRNA construct that was sprayed, indicating that the size of the dsRNA interferes with a sufficient uptake of dsRNAs by the fungus.

Schlagwörter:

Aerosols; Cytochrome P450 Family 51/genetics; disease resistance; Fungal Proteins/genetics; Fusarium/genetics; Gene Expression Regulation, Fungal/drug effects; Hordeum/growth & development/microbiology; Plant Diseases/prevention & control; RNA Interference; RNA, Double-Stranded/genetics/pharmacology

Kategorien:

1.5.3 Arabidopsis thaliana; 2.1.1 Fusarium graminearum; 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.1 endogenous; 5.1.1 stable transgene; 5.2.2 spray (SIGS); 6.3.8 metabolism; 7.2 Concept demonstration

Zeitschriftenaufsatz

Holeva, Maria C.; Sklavounos, Athanasios; Rajeswaran, Rajendran; Pooggin, Mikhail M.; Voloudakis, Andreas E. (2021):

Topical Application of Double-Stranded RNA Targeting 2b and CP Genes of Cucumber mosaic virus Protects Plants against Local and Systemic Viral Infection.

In: *Plants (Basel, Switzerland)* 10 (5). DOI: 10.3390/plants10050963.

Abstract:

Cucumber mosaic virus (CMV) is a destructive plant virus with worldwide distribution and the broadest host range of any known plant virus, as well as a model plant virus for understanding plant-virus interactions. Since the discovery of RNA interference (RNAi) as a major antiviral defense, RNAi-based technologies have been developed for plant protection against viral diseases. In plants and animals, a key trigger of RNAi is double-stranded RNA (dsRNA) processed by Dicer and Dicer-like (DCL) family proteins in small interfering RNAs (siRNAs). In the present study, dsRNAs for coat protein (CP) and 2b genes of CMV were produced in vitro and in vivo and applied onto tobacco plants representing a systemic solanaceous host as well as on a local host plant Chenopodium quinoa. Both dsRNA treatments protected plants from local and systemic infection with CMV, but not against infection with unrelated viruses, confirming sequence specificity of antiviral RNAi. Antiviral RNAi was effective when dsRNAs were applied simultaneously with or four days prior to CMV inoculation, but not four days post inoculation. In vivo-produced dsRNAs were more effective than the in vitro-produced; in treatments with in vivo dsRNAs, dsRNA-CP was more effective than dsRNA-2b, while the

effects were opposite with in vitro dsRNAs. Illumina sequencing of small RNAs from in vivo dsRNA-CP treated and non-treated tobacco plants revealed that interference with CMV infection in systemic leaves coincides with strongly reduced accumulation of virus-derived 21- and 22-nucleotide (nt) siRNAs, likely generated by tobacco DCL4 and DCL2, respectively. While the 21-nt class of viral siRNAs was predominant in non-treated plants, 21-nt and 22-nt classes accumulated at almost equal (but low) levels in dsRNA treated plants, suggesting that dsRNA treatment may boost DCL2 activity. Taken together, our findings confirm the efficacy of topical application of dsRNA for plant protection against viruses and shed more light on the mechanism of antiviral RNAi.

Schlagwörter:

cucumber mosaic virus; dsRNA; dsRNA vaccination; RNAi (RNA interference)

Kategorien:

1.1.2 Chenopodium quinoa (Quinoa); 1.2.1 Nicotiana tabacum (tobacco plant); 2.4.3 Cucumber mosaic virus (CMV); 3.3 not applicable; 4.1.2.1 viral dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2 exogenous; 6.7.7 coat protein (virus); 7.2 Concept demonstration

Zeitschriftenaufsatz

Hou, Yingnan; Zhai, Yi; Feng, Li; Karimi, Hana Z.; Rutter, Brian D.; Zeng, Liping et al. (2019):

A Phytophthora Effector Suppresses Trans-Kingdom RNAi to Promote Disease Susceptibility.

In: *Cell host & microbe* 25 (1), 153-165.e5. DOI: 10.1016/j.chom.2018.11.007.

Abstract:

RNA silencing (RNAi) has a well-established role in anti-viral immunity in plants. The destructive eukaryotic pathogen Phytophthora encodes suppressors of RNAi (PSRs), which enhance plant susceptibility. However, the role of small RNAs in defense against eukaryotic pathogens is unclear. Here, we show that Phytophthora infection of Arabidopsis leads to increased production of a diverse pool of secondary small interfering RNAs (siRNAs). Instead of regulating endogenous plant genes, these siRNAs are found in extracellular vesicles and likely silence target genes in Phytophthora during natural infection. Introduction of a plant siRNA in Phytophthora leads to developmental deficiency and abolishes virulence, while Arabidopsis mutants defective in secondary siRNA biogenesis are hypersusceptible. Notably, Phytophthora effector PSR2 specifically inhibits secondary siRNA biogenesis in Arabidopsis and promotes infection. These findings uncover the role of siRNAs as antimicrobial agents against eukaryotic pathogens and highlight a defense/counter-defense arms race centered on trans-kingdom gene silencing between hosts and pathogens.

Schlagwörter:

Arabidopsis Proteins/genetics; Arabidopsis/genetics/immunology/microbiology; Disease Susceptibility/microbiology; Gene Expression Regulation, Plant; gene silencing; Genes, Reporter/genetics; Host-Pathogen Interactions/genetics/immunology; MicroRNAs/genetics/immunology; Phosphoprotein Phosphatases/antagonists &

inhibitors/metabolism; Phytophthora/metabolism/pathogenicity; Plant Diseases/immunology/microbiology; Plant Immunity/genetics/immunology; Plant Leaves/immunology/microbiology; RNA Interference/immunology; RNA, Small Interfering/biosynthesis/drug effects/genetics; RNA-Binding Proteins/genetics/metabolism; Tobacco; Verticillium; Virulence; Virulence Factors/genetics/metabolism

Kategorien:

1.5.3 Arabidopsis thaliana; 2.1.6 Phytophthora infestans; 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.1.2.4 secondary RNAi; 4.2.2 post-transcriptional gene regulation; 5.3 not applicable; 6.9 not applicable; 7.1 Concept development

Zeitschriftenaufsatz

Hough, James; Howard, John D.; Brown, Stephen; Portwood, David E.; Kilby, Peter M.; Dickman, Mark J. (2022):

Strategies for the production of dsRNA biocontrols as alternatives to chemical pesticides.

In: *Frontiers in Bioengineering and Biotechnology* 10, S. 980592. DOI: 10.3389/fbioe.2022.980592.

Abstract:

Current crop pest control strategies rely on insecticidal and fungicidal sprays, plant genetic resistance, transgenes and agricultural practices. However, many insects, plant viruses, and fungi have no current means of control or have developed resistance against traditional pesticides. dsRNA is emerging as a novel sustainable method of plant protection as an alternative to traditional chemical pesticides. The successful commercialisation of dsRNA based biocontrols for effective pest management strategies requires the economical production of large quantities of dsRNA combined with suitable delivery methods to ensure RNAi efficacy against the target pest. A number of methods exist for the production and delivery of dsRNA based biocontrols and here we review alternative methods currently employed and emerging new approaches for their production. Additionally, we highlight potential challenges that will need to be addressed prior to widespread adoption of dsRNA biocontrols as novel sustainable alternatives to traditional chemical pesticides.

Schlagwörter:

biopesticide; dsRNA; RNAi; RNAi-based pest management

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Howard, John D.; Beghyn, Myriam; Dewulf, Nathalie; Vos, Yves de; Philips, Annelies; Portwood, David et al. (2022):

Chemically modified dsRNA induces RNAi effects in insects in vitro and in vivo: A potential new tool for improving RNA-based plant protection.

In: *The Journal of biological chemistry* 298 (9), S. 102311. DOI: 10.1016/j.jbc.2022.102311.

Abstract:

Global agriculture loses over $100 billion of produce annually to crop pests such as insects. Many of these crop pests either are not currently controlled by artificial means or have developed resistance against chemical pesticides. Long dsRNAs are capable of inducing RNAi in insects and are emerging as novel, highly selective alternatives for sustainable insect management strategies. However, there are significant challenges associated with RNAi efficacy in insects. In this study, we synthesized a range of chemically modified long dsRNAs in an approach to improve nuclease resistance and RNAi efficacy in insects. Our results showed that dsRNAs containing phosphorothioate modifications demonstrated increased resistance to southern green stink bug saliva nucleases. Phosphorothioate-modified and 2'-fluoro-modified dsRNA also demonstrated increased resistance to degradation by soil nucleases and increased RNAi efficacy in Drosophila melanogaster cell cultures. In live insects, we found chemically modified long dsRNAs successfully resulted in mortality in both stink bug and corn rootworm. These results provide further mechanistic insight into the dependence of RNAi efficacy on nucleotide modifications in the sense or antisense strand of the dsRNA in insects and demonstrate for the first time that RNAi can successfully be triggered by chemically modified long dsRNAs in insect cells or live insects.

Schlagwörter:

Animals; Drosophila melanogaster; Heteroptera/genetics; Insect Control/methods; Nucleotides/metabolism; Pest Control, Biological/methods; Pesticides/pharmacology; Plant Diseases/parasitology/prevention & control; RNA Interference; RNA, Double-Stranded/chemistry/genetics; Soil/chemistry

Kategorien:

1.6 not applicable; 2.2.2 Diabrotica virgifera virgifera (Western corn rootworm); 2.2.3 Nezara viridula (Southern green stinkbug); 2.2.4 Drosophila melanogaster; 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2.9 microinjection; 6.9 not applicable; 7.1 Concept development

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Zeitschriftenaufsatz

Hu, Dongfang; Chen, Zhi-Yuan; Zhang, Chunquan; Ganiger, Mala (2020):

Reduction of Phakopsora pachyrhizi infection on soybean through host- and spray-induced gene silencing.

In: *Molecular plant pathology* 21 (6), S. 794–807. DOI: 10.1111/mpp.12931.

Abstract:

Asian soybean rust (ASR), caused by the obligate fungal pathogen Phakopsora pachyrhizi, often leads to significant yield losses and can only be managed through fungicide applications currently. In the present study, eight urediniospore germination or appressorium formation induced P. pachyrhizi genes were investigated for their feasibility to suppress ASR through a bean pod mottle virus (BPMV)-based host-induced gene silencing (HIGS) strategy. Soybean plants expressing three of these modified BPMV vectors suppressed the expression of their corresponding target gene by 45%-80%, fungal biomass accumulation by 58%-80%, and significantly reduced ASR symptom development in soybean leaves after the plants were inoculated with P. pachyrhizi, demonstrating that HIGS can be used to manage ASR. In addition, when the in vitro synthesized double-stranded RNAs (dsRNAs) for three of the genes encoding an acetyl-CoA acyltransferase, a 40S ribosomal protein S16, and glycine cleavage system H protein were sprayed directly onto detached soybean leaves prior to P. pachyrhizi inoculation, they also resulted in an average of over 73% reduction of pustule numbers and 75% reduction in P. pachyrhizi biomass accumulation on the detached leaves compared to the controls. To the best of our knowledge, this is the first report of suppressing P. pachyrhizi infection in soybean through both HIGS and spray-induced gene silencing. It was demonstrated that either HIGS constructs targeting P. pachyrhizi genes or direct dsRNA spray application could be an effective strategy for reducing ASR development on soybean.

Schlagwörter:

Comovirus/genetics; gene silencing; Phakopsora pachyrhizi/physiology; Plant Diseases/immunology/microbiology/prevention & control; Plant Leaves/genetics/immunology/microbiology; RNA, Double-Stranded/genetics; Soybeans/genetics/immunology/microbiology

Kategorien:

1.4.6 Glycine max (soybean); 2.1.4 Phakopsora pachyrhizi (Asian soybean rust); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.1 endogenous; 5.1.1 stable transgene; 5.2.1 VIGS; 5.2.2 spray (SIGS); 6.7.6 appressorium formation; 6.8.2 urediniospore germination; 7.2 Concept demonstration

Zeitschriftenaufsatz

Hu, Jun; Xia, Yuxian (2016):

F1 -ATP synthase α-subunit: a potential target for RNAi-mediated pest management of Locusta migratoria manilensis.

In: *Pest management science* 72 (7), S. 1433–1439. DOI: 10.1002/ps.4185.

Abstract:

BACKGROUND

The migratory locust is one of the most destructive agricultural pests worldwide. ATP synthase (F0 F1 -ATPase) uses proton or sodium motive force to produce 90% of the cellular ATP, and the α-subunit of F1 -ATP synthase (ATP5A) is vital for F1 -ATP synthase. Here, we tested whether ATP5A could be a potential target for RNAi-mediated pest management of L. migratoria.

RESULTS

Lm-ATP5A was cloned and characterised. Lm-ATP5A is expressed in all tissues. Injection of 100 ng of the double-stranded RNA of ATP5A (dsATP5A) knocked down the transcription of the target gene and caused mortality in 1.5-5 days. The Lm-ATP5A protein level, the oligomycin-sensitive ATP synthetic and hydrolytic activities and the ATP content were correspondingly reduced following dsATP5A injection.

CONCLUSION

These findings demonstrated the essential roles of Lm-ATP5A in L. migratoria and identified it as a potential target for insect pest control. © 2015 Society of Chemical Industry.

Schlagwörter:

Animals; Blotting, Western; Cloning, Molecular; Gene Knockdown Techniques/methods; Insect Control/methods; Locusta migratoria/enzymology; Phylogeny; Proton-Translocating ATPases/drug effects/genetics; RNA Interference

Kategorien:

1.6 not applicable; 2.2.17 Locusta migratoria manilensis (Oriental migratory locust); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2.9 microinjection; 6.3 energy metabolism; 7.1 Concept development

Zeitschriftenaufsatz

Hu, Xu; Boeckman, Chad J.; Cong, Bin; Steimel, Joe P.; Richtman, Nina M.; Sturtz, Kristine et al. (2020):

Characterization of DvSSJ1 transcripts targeting the smooth septate junction (SSJ) of western corn rootworm (Diabrotica virgifera virgifera).

In: *Scientific reports* 10 (1), S. 11139. DOI: 10.1038/s41598-020-68014-1.

Abstract:

Transgenic maize plants expressing dsRNA targeting western corn rootworm (WCR, Diabrotica virgifera virgifera) DvSSJ1 mRNA, a Drosophila snakeskin (ssk) ortholog, show insecticidal activity and significant plant protection from WCR damage. The gene encodes a membrane protein associated with the smooth sepate junction (SSJ) which is required for intestinal barrier function. To understand the active RNA form that leads to the mortality of WCR larvae by DvSSJ1 RNA interference (RNAi), we characterized transgenic plants expressing DvSSJ1 RNA transcripts targeting WCR DvSSJ1 mRNA. The expression of the silencing cassette results in the full-length transcript of 901 nucleotides containing a 210 bp inverted fragment of the DvSSJ1 gene, the formation of a double-stranded RNA (dsRNA) transcript and siRNAs in transgenic plants. Our artificial diet-feeding study indicates that dsRNAs greater than or equal to approximately 60 base-pairs (bp) are required for DvSSJ1 insecticidal activity. Impact of specificity of dsRNA targeting DvSSJ1 mRNA on insecticidal activities was also evaluated in diet bioassay, which showed a single nucleotide mutation can have a significant impact or abolish diet activities against WCR. These results provide insights as to the functional forms of plant-delivered dsRNA for the protection of transgenic maize from WCR feeding damage and information contributing to the risk assessment of transgenic maize expressing insecticidal dsRNA.

Schlagwörter:

Animals; Coleoptera/metabolism; Insect Proteins/genetics; Intercellular Junctions/metabolism; Larva; Pest Control, Biological/methods; Plants, Genetically Modified/genetics; RNA Interference; RNA, Double-Stranded/genetics; Zea mays/genetics

Kategorien:

1.1.1 Zea mays (maize); 2.2.2 Diabrotica virgifera virgifera (Western corn rootworm); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.1 endogenous; 5.1.1 stable transgene; 6.1.13 cell-cell junctions; 7.1 Concept development

Patentschrift

Hu, Xu; Niu, Xiping; Richtman, Nina (2017):

COMPOSITIONS AND METHODS TO CONTROL INSECT PESTS.

Angemeldet durch PIONEER HI BRED INT [US] am 02.06.2017. Anmeldenr: US201716309171 20170602. Veröffentlichungsnr: US2019185867 (A1). C12N15/82. Prioriätsdaten: US201662350942P 20160616;US201716309171 20170602;WO2017US35585 20170602.

Abstract:

The present invention relates generally to methods of molecular biology and gene silencing to control pests.

Kategorien:

2.2 insects

Zeitschriftenaufsatz

Hu, Xu; Richtman, Nina M.; Zhao, Jian-Zhou; Duncan, Keith E.; Niu, Xiping; Procyk, Lisa A. et al. (2016):

Discovery of midgut genes for the RNA interference control of corn rootworm.

In: *Scientific reports* 6, S. 30542. DOI: 10.1038/srep30542.

Abstract:

RNA interference (RNAi) is a promising new technology for corn rootworm control. This paper presents the discovery of new gene targets - dvssj1 and dvssj2, in western corn rootworm (WCR). Dvssj1 and dvssj2 are orthologs of the Drosophila genes snakeskin (ssk) and mesh, respectively. These genes encode membrane proteins associated with smooth septate junctions (SSJ) which are required for intestinal barrier function. Based on bioinformatics analysis, dvssj1 appears to be an arthropod-specific gene. Diet based insect feeding assays using double-stranded RNA (dsRNA) targeting dvssj1 and dvssj2 demonstrate targeted mRNA suppression, larval growth inhibition, and mortality. In RNAi treated WCR, injury to the midgut was manifested by "blebbing" of the midgut epithelium into the gut lumen. Ultrastructural examination of midgut epithelial cells revealed apoptosis and regenerative activities. Transgenic plants expressing dsRNA targeting dvssj1 show insecticidal activity and significant plant protection from WCR damage. The data indicate that dvssj1 and dvssj2 are effective gene targets for the control of WCR using RNAi technology, by apparent suppression of production of their respective smooth septate junction membrane proteins located within the intestinal lining, leading to growth inhibition and mortality.

Schlagwörter:

Animals; Coleoptera/genetics; Gastrointestinal Tract/physiology/ultrastructure; Gene Expression Regulation; Insect Proteins/genetics; Larva/growth & development; Pest Control,

Biological/methods; Plant Roots/genetics; Plants, Genetically Modified; RNA Interference; RNA, Double-Stranded; Zea mays/genetics

Kategorien:

1.1.1 Zea mays (maize); 2.2.2 Diabrotica virgifera virgifera (Western corn rootworm); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.1 endogenous; 5.1.1 stable transgene; 5.2 exogenous; 6.1.13 cell-cell junctions; 7.2 Concept demonstration

Zeitschriftenaufsatz

Huvenne, Hanneke; Smagghe, Guy (2010):

Mechanisms of dsRNA uptake in insects and potential of RNAi for pest control: a review.

In: *Journal of insect physiology* 56 (3), S. 227–235. DOI: 10.1016/j.jinsphys.2009.10.004.

Abstract:

RNA interference already proved its usefulness in functional genomic research on insects, but it also has considerable potential for the control of pest insects. For this purpose, the insect should be able to autonomously take up the dsRNA, for example through feeding and digestion in its midgut. In this review we bring together current knowledge on the uptake mechanisms of dsRNA in insects and the potential of RNAi to affect pest insects. At least two pathways for dsRNA uptake in insects are described: the transmembrane channel-mediated uptake mechanism based on Caenorhabditis elegans' SID-1 protein and an 'alternative' endocytosis-mediated uptake mechanism. In the second part of the review dsRNA feeding experiments on insects are brought together for the first time, highlighting the achievement of implementing RNAi in insect control with the first successful experiments in transgenic plants and the diversity of successfully tested insect orders/species and target genes. We conclude with points of discussion and concerns regarding further research on dsRNA uptake mechanisms and the promising application possibilities for RNAi in insect control.

Schlagwörter:

Animals; Caenorhabditis elegans/genetics/physiology; Insect Control/methods; Insect Proteins/genetics/metabolism; Insecta/genetics/physiology; Plants, Genetically Modified/genetics/parasitology/physiology; RNA Interference; RNA, Double-Stranded/genetics/metabolism

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Islam, Md Tabibul; Davis, Zachery; Chen, Lisa; Englaender, Jacob; Zomorodi, Sepehr; Frank, Joseph et al. (2021):

Minicell-based fungal RNAi delivery for sustainable crop protection.

In: *Microbial biotechnology* 14 (4), S. 1847–1856. DOI: 10.1111/1751-7915.13699.

Abstract:

Spray-induced gene silencing (SIGS) using topical dsRNA applications has risen as a promising, target-specific, and environmentally friendly disease management strategy against phytopathogenic fungi. However, dsRNA stability, efficacy, and scalability are still the main constraints facing SIGS broader application. Here we show that Escherichia coli-derived anucleated minicells can be utilized as a cost-effective, scalable platform for dsRNA production and encapsulation. We demonstrated that minicell-encapsulated dsRNA (ME-dsRNA) was shielded from RNase degradation and stabilized on strawberry surfaces, allowing dsRNA persistence in field-like conditions. ME-dsRNAs targeting chitin synthase class III (Chs3a, Chs3b) and DICER-like proteins (DCL1 and DCL2) genes of Botryotinia fuckeliana selectively knocked-down the target genes and led to significant fungal growth inhibition in vitro. We also observed a compensatory relationship between DCL1 and DCL2 gene transcripts, where the silencing of one gene upregulated the expression of the other. Contrary to naked-dsRNAs, ME-dsRNAs halted disease progression in strawberries for 12 days under greenhouse conditions. These results elucidate the potential of ME-dsRNAs to enable the commercial application of RNAi-based, species-specific biocontrols comparable in efficacy to conventional synthetics. ME-dsRNAs offer a platform that can readily be translated to large-scale production and deployed in open-field applications to control grey mould in strawberries.

Schlagwörter:

Botrytis; crop protection; fungi; Plant Diseases/prevention & control; RNA Interference

Kategorien:

1.3.3 Fragaria sp. (strawberry); 2.1.11 Bortryotinia fuckeliana; 3.3 not applicable;
4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2.2 spray (SIGS);
5.2.4.3 minicell-encapsulated dsRNA (ME-dsRNA); 6.2.3 chitin synthesis; 7.2 Concept demonstration

Zeitschriftenaufsatz

Islam, Md Tabibul; Sherif, Sherif M. (2020):

RNAi-Based Biofungicides as a Promising Next-Generation Strategy for Controlling Devastating Gray Mold Diseases.

In: *International journal of molecular sciences* 21 (6). DOI: 10.3390/ijms21062072.

Abstract:

Botrytis cinerea is one of the most critical agro-economic phytopathogens and has been reported to cause gray mold disease in more than 1000 plant species. Meanwhile, small interfering RNA (siRNA), which induce RNA interference (RNAi), are involved in both host immunity and pathogen virulence. B. cinerea has been reported to use both siRNA effectors and host RNAi machinery to facilitate the progression of gray mold in host species. Accordingly, RNAi-based biofungicides that use double-stranded RNA (dsRNA) to target essential fungal genes are considered an emerging approach for controlling devastating gray mold diseases. Furthermore, spray-induced gene silencing (SIGS), in which the foliar application of dsRNA is used to silence the pathogen virulence genes, holds great potential as an alternative to host-induced gene silencing (HIGS). Recently, SIGS approaches have attracted research interest, owing to their ability to mitigate both pre- and post-harvest B. cinerea infections. The RNAi-mediated regulation of host immunity and susceptibility in B. cinerea-host interactions are summarized in this review, along with the limitations of the current knowledge of RNAi-based biofungicides, especially regarding SIGS approaches for controlling gray mold diseases under field conditions.

Schlagwörter:

Antifungal Agents/chemistry/pharmacology; Botrytis/genetics/growth & development; Gene Expression Regulation, Fungal/drug effects; Plant Diseases/microbiology; RNA Interference; RNA, Small Interfering/chemistry/pharmacology

Kategorien:

1.6 not applicable; 2.1.2 Botrytis cinerea; 3.3 not applicable; 4.3 not applicable; 5.2.2 spray (SIGS); 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Jin, Huihui; Abouzaid, Mostafa; Lin, Yongjun; Hull, J. Joe; Ma, Weihua (2021):

Cloning and RNAi-mediated three lethal genes that can be potentially used for Chilo suppressalis (Lepidoptera: Crambidae) management.

In: *Pesticide biochemistry and physiology* 174, S. 104828.

Abstract:

RNA interference (RNAi) has gained attention in recent years as a viable pest control strategy. Here, RNAi assays were performed to screen the potential functionality of genes in Chilo suppressalis, a serious pest of rice, and to determine their potential for developing a highly targeted molecular control approach. Potential homologs of NADH dehydrogenase (ND), glycerol 3-phosphate dehydrogenase (GPDH) and male specific lethal 3 (MSL3) were cloned from C. suppressalis, and their spatiotemporal gene expression evaluated. The expression of all three genes was higher in the pupal and adult stages than the larval stages and largely higher in the larval head compared to other tissues. Newly hatched larvae exhibited high mortalities and suppressed growth when fed bacteria producing double-stranded RNAs (dsRNAs) corresponding to the three target genes. This study provides insights into the function of ND, GPDH and MSL3 during C. suppressalis larval development and suggests that all may be candidate gene targets for C. suppressalis pest management.

Schlagwörter:

Animals; Cloning, Molecular; Genes, Lethal; Larva/genetics; Lepidoptera/genetics; Male; Moths/genetics; Oryza/genetics; RNA Interference

Kategorien:

1.1.4 Oryza sativa (rice); 2.2.9 Chilo suppressalis (Rice stem borer); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2 exogenous; 5.2.3 bmRNAi; 6.3 energy metabolism; 6.4.4 adjustment of sex specific gene expression; 7.1 Concept development

Zeitschriftenaufsatz

Kadoić Balaško, Martina; Mikac, Katarina M.; Bažok, Renata; Lemic, Darija (2020):

Modern Techniques in Colorado Potato Beetle (Leptinotarsa decemlineata Say) Control and Resistance Management: History Review and Future Perspectives.

In: *Insects* 11 (9), S. 581. DOI: 10.3390/insects11090581.

Abstract:

Colorado potato beetle, CPB (Leptinotarsa decemlineata Say), is one of the most important pests of the potato globally. Larvae and adults can cause complete defoliation of potato plant leaves and can lead to a large yield loss. The insect has been successfully suppressed by insecticides; however, over time, has developed resistance to insecticides from various chemical groups, and its once successful control has diminished. The number of available active chemical control substances is decreasing with the process of testing, and registering new products on the market are time-consuming and expensive, with the possibility of resistance ever present. All of these concerns have led to the search for new methods to control CPB and efficient tools to assist with the detection of resistant variants and monitoring of resistant populations. Current strategies that may aid in slowing resistance include gene silencing by RNA interference (RNAi). RNAi, besides providing an efficient tool for gene functional studies, represents a safe, efficient, and eco-friendly strategy for CPB control. Genetically

modified (GM) crops that produce the toxins of Bacillus thuringiensis (Bt) have many advantages over agro-technical, mechanical, biological, and chemical measures. However, pest resistance that may occur and public acceptance of GM modified food crops are the main problems associated with Bt crops. Recent developments in the speed, cost, and accuracy of next generation sequencing are revolutionizing the discovery of single nucleotide polymorphisms (SNPs) and field of population genomics. There is a need for effective resistance monitoring programs that are capable of the early detection of resistance and successful implementation of integrated resistance management (IRM). The main focus of this review is on new technologies for CPB control (RNAi) and tools (SNPs) for detection of resistant CPB populations.

Schlagwörter:

CPB - Colorado Potato Beetle; Pest Control; potato (Solanum tuberosum); resistance problem; RNAi (RNA interference); SNP (single nucleotide polymorphism)

Kategorien:

1.4.5 Solanum tuberosum (potato); 2.2.19 Leptinotarsa decemlineata (Colorado potato beetle); 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Khajuria, Chitvan; Ivashuta, Sergey; Wiggins, Elizabeth; Flagel, Lex; Moar, William; Pleau, Michael et al. (2018):

Development and characterization of the first dsRNA-resistant insect population from western corn rootworm, Diabrotica virgifera virgifera LeConte.

In: *PloS one* 13 (5), e0197059. DOI: 10.1371/journal.pone.0197059.

Abstract:

The use of dsRNA to control insect pests via the RNA interference (RNAi) pathway is being explored by researchers globally. However, with every new class of insect control compounds, the evolution of insect resistance needs to be considered, and understanding resistance mechanisms is essential in designing durable technologies and effective resistance management strategies. To gain insight into insect resistance to dsRNA, a field screen with subsequent laboratory selection was used to establish a population of DvSnf7 dsRNA-resistant western corn rootworm, Diabrotica virgifera virgifera, a major maize insect pest. WCR resistant to ingested DvSnf7 dsRNA had impaired luminal uptake and resistance was not DvSnf7 dsRNA-specific, as indicated by cross resistance to all other dsRNAs tested. No resistance to the Bacillus thuringiensis Cry3Bb1 protein was observed. DvSnf7 dsRNA resistance was inherited recessively, located on a single locus, and autosomal. Together these findings will provide insights for dsRNA deployment for insect pest control.

Schlagwörter:

Animals; Animals, Genetically Modified/genetics; Coleoptera/genetics; Pest Control, Biological; RNA, Double-Stranded/genetics; Zea mays/parasitology

Kategorien:

1.1.1 Zea mays (maize); 2.2.2 Diabrotica virgifera virgifera (Western corn rootworm); 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.1 Concept development

Zeitschriftenaufsatz

Knorr, Eileen; Bingsohn, Linda; Kanost, Michael R.; Vilcinskas, Andreas (2013):

Tribolium castaneum as a model for high-throughput RNAi screening.

In: *Advances in biochemical engineering/biotechnology* 136, S. 163–178. DOI: 10.1007/10_2013_208.

Abstract:

Coleopteran insects are a highly diverse and successful order, and many beetle species are significant agricultural pests. New biorational strategies for managing populations of beetles and other insect species are needed as pests develop resistance to chemical insecticides and Bt toxins. There is now an opportunity to use genome sequence data to identify genes that are essential for insect growth, development, or survival as new targets for designing control technology. This goal requires a method for high-throughput in vivo screening of thousands of genes to identify candidate genes that, when their expression is disrupted, have a phenotype that may be useful in insect pest control. Tribolium castaneum, the red flour beetle, is a model organism that offers considerable advantages for such screening, including ease of rearing in large numbers, a sequenced genome, and a strong, systemic RNAi response for specific depletion of gene transcripts. The RNAi effect in T. castaneum can be elicited in any tissue and any stage by the injection of dsRNA into the hemocoel, and injection of dsRNA into adult females can even be used to identify phenotypes in offspring. A pilot RNAi screen (iBeetle) is underway. Several T. castaneum genes with promising RNAi phenotypes for further development as mechanisms for plant protection have been identified. These include heat shock protein 90, chitin synthase, the segmentation gene hairy, and a matrix metalloprotease. Candidate genes identified in T. castaneum screens can then be tested in agricultural pest species (in which screening is not feasible), to evaluate their effectiveness for use in potential plant-based RNAi control strategies. Delivery of dsRNA expressed by genetically modified crops to the midgut of phytophagous insects is under investigation as a new tool for very specific protection of plants from insect pest species. The T. castaneum screening platform offers a system for discovery of candidate genes with high potential benefit.

Schlagwörter:

Animals; High-Throughput Nucleotide Sequencing/methods; Insect Control/methods; Insect Proteins/genetics; Pest Control, Biological/methods; Plants, Genetically Modified/genetics/parasitology; RNA Interference/physiology; Tribolium/genetics

Kategorien:

1.6 not applicable; 2.2.12 Tribolium castaneum (Red flour beetle); 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Koch, Aline; Biedenkopf, Dagmar; Furch, Alexandra; Weber, Lennart; Rossbach, Oliver; Abdellatef, Eltayb et al. (2016):

An RNAi-Based Control of Fusarium graminearum Infections Through Spraying of Long dsRNAs Involves a Plant Passage and Is Controlled by the Fungal Silencing Machinery.

In: *PLoS pathogens* 12 (10), e1005901. DOI: 10.1371/journal.ppat.1005901.

Abstract:

Meeting the increasing food and energy demands of a growing population will require the development of ground-breaking strategies that promote sustainable plant production. Host-induced gene silencing has shown great potential for controlling pest and diseases in crop plants. However, while delivery of inhibitory noncoding double-stranded (ds)RNA by transgenic expression is a promising concept, it requires the generation of transgenic crop plants which may cause substantial delay for application strategies depending on the transformability and genetic stability of the crop plant species. Using the agronomically important barley-Fusarium graminearum pathosystem, we alternatively demonstrate that a spray application of a long noncoding dsRNA (791 nt CYP3-dsRNA), which targets the three fungal cytochrome P450 lanosterol C-14α-demethylases, required for biosynthesis of fungal ergosterol, inhibits fungal growth in the directly sprayed (local) as well as the non-sprayed (distal) parts of detached leaves. Unexpectedly, efficient spray-induced control of fungal infections in the distal tissue involved passage of CYP3-dsRNA via the plant vascular system and processing into small interfering (si)RNAs by fungal DICER-LIKE 1 (FgDCL-1) after uptake by the pathogen. We discuss important consequences of this new finding on future RNA-based disease control strategies. Given the ease of design, high specificity, and applicability to diverse pathogens, the use of target-specific dsRNA as an anti-fungal agent offers unprecedented potential as a new plant protection strategy.

Schlagwörter:

Biological Control Agents/administration & dosage; Blotting, Northern; Fusariosis/prevention & control; Hordeum/genetics/parasitology; Microscopy, Confocal; Pest Control, Biological/methods; Plant Diseases/prevention & control; Plants, Genetically Modified; RNA Interference; RNA, Double-Stranded/administration & dosage; RNA, Small Interfering/administration & dosage

Kategorien:

1.1.5 Hordeum vulgare (barley); 2.1.1 Fusarium graminearum; 3.3 not applicable; 4.1.2.2 long non-coding dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2.2 spray (SIGS); 6.1.11 ergosterol biosynthesis; 7.2 Concept demonstration

Zeitschriftenaufsatz

Koch, Aline; Höfle, Lisa; Werner, Bernhard Timo; Imani, Jafargholi; Schmidt, Alexandra; Jelonek, Lukas; Kogel, Karl-Heinz (2019):

SIGS vs HIGS: a study on the efficacy of two dsRNA delivery strategies to silence Fusarium FgCYP51 genes in infected host and non-host plants.

In: *Molecular plant pathology* 20 (12), S. 1636–1644. DOI: 10.1111/mpp.12866.

Abstract:

CYP3RNA, a double-stranded (ds)RNA designed to concomitantly target the two sterol 14α-demethylase genes FgCYP51A and FgCYP51B and the fungal virulence factor FgCYP51C, inhibits the growth of the ascomycete fungus Fusarium graminearum (Fg) in vitro and in planta. Here we compare two different methods (setups) of dsRNA delivery, viz. transgene expression (host-induced gene silencing, HIGS) and spray application (spray-induced gene silencing, SIGS), to assess the activity of CYP3RNA and novel dsRNA species designed to target one or two FgCYP51 genes. Using Arabidopsis and barley, we found that dsRNA designed to target two FgCYP51 genes inhibited fungal growth more efficiently than dsRNA targeting a single gene, although both dsRNA species reduced fungal infection. Either dsRNA delivery method reduced fungal growth stronger than anticipated from previous mutational knock-out (KO) strategies, where single gene KO had no significant effect on fungal viability. Consistent with the strong inhibitory effects of the dsRNAs on fungal development in both setups, we detected to a large extent dsRNA-mediated co-silencing of respective non-target FgCYP51 genes. Together, our data further support the valuation that dsRNA applications have an interesting potential for pesticide target validation and gene function studies, apart from their potential for crop protection.

Schlagwörter:

Arabidopsis/microbiology; Cytochrome P-450 Enzyme System/genetics; Fusarium/drug effects/genetics; gene silencing; Gene Targeting/methods; Genes, Fungal/drug effects; Hordeum/microbiology; Plant Diseases/microbiology; RNA, Double-Stranded/pharmacology; Software; Transgenes

Kategorien:

1.1.5 Hordeum vulgare (barley); 1.5.3 Arabidopsis thaliana; 2.1.1 Fusarium graminearum; 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.1 endogenous; 5.1.1 stable transgene; 5.2 exogenous; 5.2.2 spray (SIGS); 6.1.11 ergosterol biosynthesis; 6.7.8 infection mechanisms (virulence factors); 7.2 Concept demonstration

Zeitschriftenaufsatz

Koch, Aline; Kogel, Karl-Heinz (2014):

New wind in the sails: improving the agronomic value of crop plants through RNAi-mediated gene silencing.

In: *Plant Biotechnol J* 12 (7), S. 821–831. DOI: 10.1111/pbi.12226.

Abstract:

RNA interference (RNAi) has emerged as a powerful genetic tool for scientific research over the past several years. It has been utilized not only in fundamental research for the assessment of gene function, but also in various fields of applied research, such as human and veterinary medicine and agriculture. In plants, RNAi strategies have the potential to allow manipulation of various aspects of food quality and nutritional content. In addition, the demonstration that agricultural pests, such as insects and nematodes, can be killed by exogenously supplied RNAi targeting their essential genes has raised the possibility that plant predation can be controlled by lethal RNAi signals generated in planta. Indeed, recent evidence argues that this strategy, called host-induced gene silencing (HIGS), is effective against sucking insects and nematodes; it also has been shown to compromise the growth and development of pathogenic fungi, as well as bacteria and viruses, on their plant hosts. Here, we review recent studies that reveal the enormous potential RNAi strategies hold not only for improving the nutritive value and safety of the food supply, but also for providing an environmentally friendly mechanism for plant protection.

Schlagwörter:

breeding; Crops, Agricultural/genetics/microbiology/parasitology; food safety; Food Supply; Gene Expression Regulation, Plant; Host-Pathogen Interactions; Models, Genetic; Nutritive Value/genetics; Pest Control, Biological; RNA Interference

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Koch, Aline; Kumar, Neelendra; Weber, Lennart; Keller, Harald; Imani, Jafargholi; Kogel, Karl-Heinz (2013):

Host-induced gene silencing of cytochrome P450 lanosterol C14α-demethylase-encoding genes confers strong resistance to Fusarium species.

In: *Proceedings of the National Academy of Sciences of the United States of America* 110 (48), S. 19324–19329. DOI: 10.1073/pnas.1306373110.

Abstract:

Head blight, which is caused by mycotoxin-producing fungi of the genus Fusarium, is an economically important crop disease. We assessed the potential of host-induced gene silencing targeting the fungal cytochrome P450 lanosterol C-14α-demethylase (CYP51) genes, which are essential for ergosterol biosynthesis, to restrict fungal infection. In axenic cultures of Fusarium graminearum, in vitro feeding of CYP3RNA, a 791-nt double-stranded (ds)RNA complementary to CYP51A, CYP51B, and CYP51C, resulted in growth inhibition [half-maximum growth inhibition (IC50) = 1.2 nM] as well as altered fungal morphology, similar to that observed after treatment with the azole fungicide tebuconazole, for which the CYP51 enzyme is a target. Expression of the same dsRNA in Arabidopsis and barley rendered susceptible plants highly resistant to fungal infection. Microscopic analysis revealed that mycelium formation on CYP3RNA-expressing leaves was restricted to the inoculation sites, and that inoculated barley caryopses were virtually free of fungal hyphae. This inhibition of fungal growth correlated with in planta production of siRNAs corresponding to the targeted CYP51 sequences, as well as highly efficient silencing of the fungal CYP51 genes. The high efficiency of fungal inhibition suggests that host-induced gene-silencing targeting of the CYP51 genes is an alternative to chemical treatments for the control of devastating fungal diseases.

Schlagwörter:

Arabidopsis/microbiology; Disease Resistance/genetics; Fusarium/enzymology/genetics; gene silencing; Hordeum/microbiology; Plant Diseases/microbiology; Plants, Genetically Modified; RNA, Double-Stranded/metabolism; Sterol 14-Demethylase/genetics

Kategorien:

1.1.5 Hordeum vulgare (barley); 1.5.3 Arabidopsis thaliana; 2.1.7 Fusarium spp.; 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.1 endogenous; 5.1.1 stable transgene; 6.1.11 ergosterol biosynthesis; 7.2 Concept demonstration

Zeitschriftenaufsatz

Koch, Aline; Wassenegger, Michael (2021):

Host-induced gene silencing - mechanisms and applications.

In: *The New phytologist* 231 (1), S. 54–59. DOI: 10.1111/nph.17364.

Abstract:

Host-induced gene silencing (HIGS) technology has emerged as a powerful alternative to chemical treatments for protecting plants from pathogens or pests. More than 170 HIGS studies have been published so far, and HIGS products have been launched. First, we discuss the strengths and limitations of this technology in a pathosystem-specific context. Next, we highlight the requirement for fundamental knowledge on the molecular mechanisms (i.e. uptake, processing and translocation of transgene-expressed double-stranded RNAs) that determine the efficacy and specificity of HIGS. Additionally, we speculate on the contribution of host and target RNA interference machineries, which may be incompatible depending on the lifestyle of the pathogen or pest. Finally, we predict that closing these gaps in knowledge will lead to the development of novel integrative concepts, precise risk assessment and tailor-made HIGS therapy for plant diseases.

Schlagwörter:

dsRNA; extracellular vesicles; gene silencing; HIGS (host-induced gene silencing); Plant Diseases/genetics; Plants/genetics; RNA Interference; RNA, Double-Stranded; RNAi (RNA interference); RNAi-based plant protection; siRNA

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.1 endogenous; 5.1.1 stable transgene; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Koeppe, Sarah; Kawchuk, Lawrence; Kalischuk, Melanie (2023):

RNA Interference Past and Future Applications in Plants.

In: *International journal of molecular sciences* 24 (11). DOI: 10.3390/ijms24119755.

Abstract:

Antisense RNA was observed to elicit plant disease resistance and post-translational gene silencing (PTGS). The universal mechanism of RNA interference (RNAi) was shown to be induced by double-stranded RNA (dsRNA), an intermediate produced during virus replication. Plant viruses with a single-stranded positive-sense RNA genome have been instrumental in the discovery and characterization of systemic RNA silencing and suppression. An increasing number of applications for RNA silencing have emerged involving the exogenous application of dsRNA through spray-induced gene silencing (SIGS) that provides specificity and environmentally friendly options for crop protection and improvement.

Schlagwörter:

gene silencing; Plant Diseases; Plants/genetics; RNA Interference; RNA, Double-Stranded/genetics; RNA, Small Interfering/genetics

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.2.2 spray (SIGS); 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Kolge, Henry; Kadam, Kartiki; Galande, Sharad; Lanjekar, Vikram; Ghormade, Vandana (2021):

New Frontiers in Pest Control: Chitosan Nanoparticles-Shielded dsRNA as an Effective Topical RNAi Spray for Gram Podborer Biocontrol.

In: *ACS applied bio materials* 4 (6), S. 5145–5157. DOI: 10.1021/acsabm.1c00349.

Abstract:

Chickpea pod borer, Helicoverpa armigera, displays resistance to chemical insecticides and transgenics. The potential nontransformative RNAi approach of specific gene silencing by mRNA breakdown through exogenous double-stranded (dsRNA) delivery to Helicoverpa faces problems of degradation by nucleases and insect gut pH. We demonstrate that chitosan nanoparticles (CNPs) effectively mediate specific dsRNA delivery against Helicoverpa armigerajuvenile hormone methyltransferase (JHAMT) and acetylcholine esterase (ACHE) target genes. Ionotropically synthesized cationic CNPs (100 nm size, +32 mV charge) loaded dsRNA efficiently and protected it effectively from degradation by nucleases and insect gut pH. Tagging CNPs with Calcofluor fluorescence illustrated its efficient uptake in columnar insect gut cells. The potential of CNPs-mediated dsRNA delivery was elucidated with effective silencing of green fluorescent protein transformed Sf9 cells. Furthermore, CNPs-dsRNA complexes were stable for 5 d on leaf surfaces, and their ingestion with leaf effectively silenced H. armigeraJHAMT and ACHE genes to suppress related enzyme activities and caused 100% insect mortality. Further, in planta bioassay with CNPs-dsRNA spray confirmed the RNAi induced insect mortality. Moreover, CNPs-dsRNA fed nontarget insects Spodoptera litura and Drosophila melanogaster were unaffected, and no toxicity was observed for CNPs in cell line studies. Remarkably, only two low dose (0.028 g/ha) topical CNPs-ache-dsRNA sprays on chickpea displayed reduced pod damage with high yields on par with chemical control in the field, which was followed by CNPs-jhamt-dsRNA nanoformulation. These studies can pave the way for the development of topical application of CNPs-dsRNA spray as a safe, specific, innovative insecticide for sustainable crop protection.

Schlagwörter:

Animals; Chitosan/pharmacology; Drosophila melanogaster/genetics; Insecta/genetics; Insecticides/pharmacology; Juvenile Hormones; Moths/genetics; Nanoparticles; RNA Interference; RNA, Double-Stranded/genetics

Kategorien:

1.4.7 Cicer aestivum (chickpea); 2.2.1 Helicoverpa armigera (Cotton bollworm/Chickpea pod borer); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2.1 mRNA degradation; 5.2.4.1 nanoparticle-mediated dsRNA; 6.2.4 molting; 6.2.5.1 metamorphosis; 6.6 neuronal system; 7.1 Concept development

Zeitschriftenaufsatz

Koutouleas, Athina; Collinge, David B.; Ræbild, Anders (2023):

Alternative plant protection strategies for tomorrow's coffee.

In: *Plant Pathology* 72 (3), S. 409–429. DOI: 10.1111/ppa.13676.

Abstract:

Continuous pesticide usage has negative impacts on people and ecosystems associated with coffee farms. Alternative plant protection strategies can be implemented that are sustainable for both the environment and the coffee farmer. In this review, new genomic techniques (NGTs) such as RNAi (RNA interference, using spray--induced gene silencing –- SIGS) are presented as a possible novel strategy to manage *Coffea arabica* pests and diseases. Exploitation of the coffee agroforestry system (AFS) is presented as another strategy, offering both plant protection and ecosystem restoration

functions. Interactions within a coffee--AFS were found to both hinder and bolster the development of some coffee pests and diseases. Biological control represents a third strategy that has been examined to--date to combat important coffee pests and diseases (i.e., American leaf spot, black coffee twig borer, coffee berry borer, coffee berry disease, coffee leaf miner, coffee leaf rust, coffee wilt disease and green coffee scale). The astute use of RNAi, AFS and/or biological control have the potential to provide alternatives to conventional pesticides for future sustainable coffee production. However, these approaches must be compatible with the coffee farmers' local needs and accessibility and bolstered through nationwide support by advisory services and coffee authorities.

Schlagwörter:

agroforestry; Coffea arabica; Pest Control, Biological; Pesticides; RNAi; smallholder farmer

Kategorien:

1.5.5 Coffea arabica; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Kwon, Deok Ho; Park, Ji Hyun; Lee, Si Hyeock (2017):

Identification of initial responsive genes to systemic dsRNA ingestion in the two-spotted spider mite, Tetranychus urticae Koch.

In: *Journal of Asia-Pacific Entomology* 20 (1), S. 229–235. DOI: 10.1016/j.aspen.2017.01.006.

Abstract:

Ingestion of double-stranded RNA (dsRNA) is known to induce RNA interference (RNAi) in the two-spotted spider mite, Tetranychus urticae, but little is known about the underlying molecular processes. To better understand the molecular mechanisms involved in ingestion-mediated RNAi, we compared transcriptomic profiles to identify genes that respond to the T. urticae-specific (dsCOPB2) or -non-specific (dsEGFP) dsRNA. In total, 450 and 296 genes were differentially expressed in response to dsCOPB2 and dsEGFP, respectively ($P < 0.05$). Among them, 180 genes commonly responded to both dsCOPB2 and dsEGFP, and their expression patterns were highly correlated between the two dsRNAs ($r = 0.892$, $P < 0.001$). Gene ontology analysis revealed that proteins associated with 'metabolic process' (76.2%), 'catalytic activity' (64.9%), and 'membrane' (63%) occupied the highest proportion within the categories of 'biological process', 'molecular function', and 'cellular process', respectively. The proportions of down-regulated genes were 2.2- and 2.5-fold greater than those of up-regulated genes in the categories of 'cellular process' and 'molecular function', respectively, whereas the proportion of up-regulated genes was 1.7-fold greater in the cellular component category. Interestingly, the genes that were the most highly differentially expressed following dsRNA ingestion were found to encode metabolic proteins with various catalytic functions, such as cathepsin, intradiol ring-cleavage dioxygenase, carboxylesterase, serine protease, and UDP-glycosyltransferase. Genes associated with dsRNA uptake and RNAi machinery were only rarely identified, suggesting that genes associated with dsRNA uptake and the RNAi process may not be actively engaged at 36 h post-treatment, the observation time in this study. Our results suggest that metabolic enzymes poisessing various types of catalytic activity appear to function before the activation of RNAi machinery in T. urticae.

Schlagwörter:

dsRNA; RNAi (RNA interference); rna-seq; Tetranychus urticae; transcriptome analysis

Kategorien:

1.6 not applicable; 2.5.1 Tetranychus urticae; 3.2 Effect; 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.3 not applicable; 6.1.6 membrane; 6.3.6 catalytic activity; 6.3.8 metabolism; 7.1 Concept development

Zeitschriftenaufsatz

Langenbach, Caspar; Campe, Ruth; Beyer, Sebastian F.; Mueller, André N.; Conrath, Uwe (2016):

Fighting Asian Soybean Rust.

In: *Frontiers in Plant Science* 7, S. 797. DOI: 10.3389/fpls.2016.00797.

Abstract:

Phakopsora pachyrhizi is a biotrophic fungus provoking SBR disease. SBR poses a major threat to global soybean production. Though several R genes provided soybean immunity to certain P. pachyrhizi races, the pathogen swiftly overcame this resistance. Therefore, fungicides are the only current means to control SBR. However, insensitivity to fungicides is soaring in P. pachyrhizi and, therefore, alternative measures are needed for SBR control. In this article, we discuss the different approaches for fighting SBR and their potential, disadvantages, and advantages over other measures. These encompass conventional breeding for SBR resistance, transgenic approaches, exploitation of transcription factors, secondary metabolites, and antimicrobial peptides, RNAi/HIGS, and biocontrol strategies. It seems that an integrating approach exploiting different measures is likely to provide the best possible means for the effective control of SBR.

Schlagwörter:

Asian soybean rust; fungicide insensitivity; host resistance; non-host resistance; Phakopsora pachyrhizi; plant biotechnology; plant breeding

Kategorien:

1.4.6 Glycine max (soybean); 2.1.4 Phakopsora pachyrhizi (Asian soybean rust); 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Lee, Dae-Weon; Kim, Yonggyun; Koh, Young Ho (2011):

RNA interference of PBAN receptor suppresses expression of two fatty acid desaturases in female Plutella xylostella.

In: *Journal of Asia-Pacific Entomology* 14 (4), S. 405–410. DOI: 10.1016/j.aspen.2011.05.004.

Abstract:

Pheromone biosynthesis-activating neuropeptide (PBAN) stimulates sex pheromone biosynthesis by activating PBAN receptor (PBANr), which triggers a specific signal transduction in the pheromone gland cells. We have shown that RNA interference (RNAi) of PBANr of Plutella xylostella significantly suppressed pheromone biosynthesis and subsequent mating behavior. In order to assess molecular events occurring downstream of PBAN signaling, we cloned partial sequences of Delta 9 and Delta 11 fatty acid desaturases of P. xylostella. Phylogenetic analysis indicated that these two desaturase genes were highly clustered with other desaturases associated with sex pheromone biosynthesis in other insects. RT-PCR analysis showed that Delta 9 desaturase was dominantly expressed in adult females, whereas Delta 11 desaturase was expressed in all P. xylostella developmental stages. When PBANr expression was suppressed by PBANr-RNAi, the treated females also showed significant suppression of expression of both desaturases. These results suggest that expressions of the two desaturases are controlled by PBAN and that the two desaturases may be involved as downstream components in sex pheromone biosynthesis of P. xylostella. (C) Korean Society of Applied Entomology, Taiwan Entomological Society and Malaysian Plant Protection Society, 2011. Published by Elsevier B.V.

Schlagwörter:

desaturases; PBAN receptor; Plutella xylostella; RNAi (RNA interference); sex pheromone

Kategorien:

1.6 not applicable; 2.2.11 Plutella xylostella (Diamondback moth); 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.4.3 pheromon production/mating behaviour; 7.1 Concept development

Zeitschriftenaufsatz

Li, Daqi; Zhang, Jianqin; Yang, Yang; Liu, Jiao; Lu, Junjiao; Ren, Meifeng et al. (2022):

Identification and RNAi-based functional analysis of chitinase family genes in Agrotis ipsilon.

In: *Pest management science* 78 (10), S. 4278–4287. DOI: 10.1002/ps.7047.

Abstract:

BACKGROUND

Chitin is a major component in the extracellular matrix of insects, and its metabolism largely affects insect development and molting. As essential degradative enzymes, chitinases are encoded by multiple genes that differ in size, expression pattern and function in insects. However, our limited knowledge on the functions of different chitinases in Agrotis ipsilon has prevented our application of new technologies to target these genes as new pest management strategies.

RESULTS

We revealed 11 full-length complementary DNA sequences of chitinase genes (AiChts) from A. ipsilon transcriptome. Although the domain architecture of these chitinases varied greatly, they all contained at least one chitinase catalytic domain. Developmental stage- and tissue-dependent expression profiles showed that most AiChts had the highest expression in the pupal stage.

Furthermore, AiCht2, AiCht6, AiCht7 and AiCht10 were mainly expressed in the integument, whereas AiCht8 and AiCht-h had the highest expression in the midgut. The RNA interference (RNAi) experiment revealed that knockdown of AiCht10 or the imaginal disc growth factor gene (AiIDGF) induced high larval mortality. Larvae failed to shed the old cuticle during molting after the injection of double-stranded RNA targeting AiCht10 (dsAiCht10), whereas the larval bodies shrunk and blackened after the injection of dsRNA targeting AiIDGF (dsAiIDGF).

CONCLUSION

Our results revealed for the first time the important functions of AiCht10 and AiIDGF in A. ipsilon. These genes are essential for larval development, and can potentially serve as new targets for RNAi-based pest management. © 2022 Society of Chemical Industry.

Schlagwörter:

Animals; Chitinases/genetics; Insect Proteins/genetics/metabolism; Larva; Molting/genetics; Moths; Pupa; RNA Interference; RNA, Double-Stranded/genetics

Kategorien:

1.6 not applicable; 2.2.6 Agrotis ipsilon; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.2.3 chitin synthesis; 6.2.4 molting; 6.2.5.1 metamorphosis; 7.1 Concept development

Zeitschriftenaufsatz

Li, H.; Khajuria, C.; Rangasamy, M.; Gandra, P.; Fitter, M.; Geng, C. et al. (2015):

Long dsRNA but not siRNA initiates RNAi in western corn rootworm larvae and adults.

In: *J Appl Entomol* 139 (6), S. 432–445. DOI: 10.1111/jen.12224.

Abstract:

Transgenic maize plants expressing dsRNA targeting western corn rootworm (WCR, Diabrotica virgifera virgifera LeConte) v-ATPase subunit C mRNA for RNAi provided significant root protection from WCR larval feeding damage in greenhouse assays compared to negative controls. Transcribed hairpin dsRNA in WCR-resistant maize plants was present as both intact hairpin-derived dsRNA and plant-processed siRNA. Therefore, the ability of dsRNA and siRNA targeting Dv v-ATPase CmRNA to cause an RNAi response was studied in both WCR larvae and adults. In 9-day diet-based feeding assays, dsRNA of at least 60 bp in length resulted in high levels of larval mortality. In contrast, 15-, 25- or 27-bp dsRNAs or pooled 21-bp siRNAs did not cause mortality of exposed larvae. When larvae were fed with diet overlaid with siRNAs, Dv v-ATPase C transcript levels did not change. Conversely, when WCR larvae were fed with diet overlaid with 184-bp dsRNA, the mRNA level was reduced by >20-fold relative to yfp dsRNA negative control. Similarly, 184-bp dsRNA caused 100% mortality of WCR adults, whereas the mortality of adults fed on diet treated with siRNAs was similar to the negative control. Feeding adults with siRNAs on diet did not affect the level of Dv v-ATPase CmRNA transcripts, whereas adults fed with the 184-bp dsRNA showed approximately 35-fold reduction in the target mRNA level. Similar results were obtained with the WCR adults injected with 184-bp dsRNA or 21-bp siRNA. These results suggest that only long dsRNA or hairpin-derived dsRNA is effective in causing lethal knock-down of Dv v-ATPase CmRNA. These results have implications for efficacious plant-delivered dsRNA for the protection of transgenic maize from WCR feeding damage and for the risk assessment of transgenic maize expressing insecticidal dsRNA.

Schlagwörter:

Diabrotica virgifera virgifera; dsRNA; siRNA; transgenic RNA maize; v-ATPase subunit C; western corn rootworm

Kategorien:

1.1.1 Zea mays (maize); 2.2.2 Diabrotica virgifera virgifera (Western corn rootworm); 3.3 not applicable; 4.1.1 shRNA; 4.2.2 post-transcriptional gene regulation; 4.2.2.1 mRNA degradation; 5.1 endogenous; 5.1.1 stable transgene; 6.3 energy metabolism; 7.2 Concept demonstration

Zeitschriftenaufsatz

Li, Jianhao; Qian, Jin; Xu, Yuanyuan; Yan, Shuo; Shen, Jie; Yin, Meizhen (2019):

A Facile-Synthesized Star Polycation Constructed as a Highly Efficient Gene Vector in Pest Management.

In: *ACS Sustainable Chem. Eng.* 7 (6), S. 6316–6322. DOI: 10.1021/acssuschemeng.9b00004.

Abstract:

Gene vectors have been extensively applied in various fields. However, the high economic cost of gene vectors limits their development and application, and there is an urgent demand for developing highly efficient gene vectors with low cost, especially for large-scale application. Here, we reported a simple but effective star polycation as a gene vector for pest management. Our polycation was constructed by commercial and cheap material sources, and the facile synthesis procedure was simplified to two reaction steps, decreasing the cost to a large extent. This vector showed a low cytotoxicity as well as a high gene delivery efficacy into live cells. The vector-doublestranded RNA (dsRNA) down-regulated the pest key developmental gene expression to inhibit the pest growth. Our work provides an efficient gene vector with low cost for scientific researches, which may also promote the practice and development of RNA interference (RNAi)-based pest management.

Schlagwörter:

facile synthesized; gene vector; low cost; RNAi-based pest management; star polycation

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.1.2.3 star polycation gene vector dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2 exogenous; 5.2.4 carrier; 5.2.4.7 star polycation; 6.9 not applicable; 7.2 Concept demonstration

Zeitschriftenaufsatz

Li, Tengchao; Chen, Jie; Fan, Xiaobin; Chen, Weiwen; Zhang, Wenqing (2017):

MicroRNA and dsRNA targeting chitin synthase A reveal a great potential for pest management of the hemipteran insect Nilaparvata lugens.

In: *Pest management science* 73 (7), S. 1529–1537. DOI: 10.1002/ps.4492.

Abstract:

BACKGROUND

Two RNA silencing pathways in insects are known to exist that are mediated by short interfering RNAs (siRNAs) and microRNAs (miRNAs), which have been hypothesised to be promising methods for insect pest control. However, a comparison between miRNA and siRNA in pest control is still unavailable, particularly in targeting chitin synthase gene A (CHSA).

RESULTS

The dsRNA for Nilaparvata lugens CHSA (dsNlCHSA) and the microR-2703 (miR-2703) mimic targeting NlCHSA delivered via feeding affected the development of nymphs, reduced their chitin content and led to lethal phenotypes. The protein level of NlCHSA was downregulated after female adults were injected with dsNlCHSA or the miR-2703 mimic, but there were no significant differences in vitellogenin (NlVg) expression or in total oviposition relative to the control group. However, 90.68 and 46.13% of the eggs laid by the females injected with dsNlCHSA and miR-2703 mimic were unable to hatch, respectively. In addition, a second-generation miRNA and RNAi effect on N. lugens was observed.

CONCLUSION

Ingested miR-2703 seems to be a good option for killing N. lugens nymphs, while NlCHSA may be a promising target for RNAi-based pest management. These findings provide important evidence for applications of small non-coding RNAs (snRNAs) in insect pest management. © 2016 Society of Chemical Industry.

Schlagwörter:

Animals; Chitin Synthase/genetics; Chitin/biosynthesis/genetics; Female; Hemiptera/enzymology/genetics/growth & development; Insect Control/methods; Insect Proteins/genetics/metabolism; MicroRNAs; Nymph/growth & development; RNA Interference; RNA, Double-Stranded/genetics/metabolism; RNA, Small Interfering

Kategorien:

1.6 not applicable; 2.2.14 Nilaparvata lugens (Brown planthopper); 3.3 not applicable; 4.1.1.1 miRNA; 4.2.2 post-transcriptional gene regulation; 5.2 exogenous; 6.2.3 chitin synthesis; 7.1 Concept development

Zeitschriftenaufsatz

Li, Xiang; Liu, Xiaoguang; Lu, Wenhui; Yin, Xinming; An, Shiheng (2022):

Application progress of plant-mediated RNAi in pest control.

In: *Frontiers in Bioengineering and Biotechnology* 10, S. 963026. DOI: 10.3389/fbioe.2022.963026.

Abstract:

RNA interference (RNAi)-based biopesticides are novel biologic products, developed using RNAi principles. They are engineered to target genes of agricultural diseases, insects, and weeds, interfering with their target gene expression so as to hinder their growth and alleviate their damaging effects on crops. RNAi-based biopesticides are broadly classified into resistant plant-based plant-incorporated protectants (PIPs) and non-plant-incorporated protectants. PIP RNAi-based biopesticides are novel biopesticides that combine the advantages of RNAi and resistant transgenic crops. Such RNAi-based biopesticides are developed through nuclear or plastid transformation to breed resistant plants, i.e., dsRNA-expressing transgenic plants. The dsRNA of target genes is expressed in the plant cell, with pest and disease control being achieved through plant-target organism interactions. Here, we review the action mechanism and strategies of RNAi for pest management, the development of RNAi-based transgenic plant, and the current status and advantages of deploying these products for pest control, as well as the future research directions and problems in production and commercialization. Overall, this study aims to elucidate the current development status of RNAi-based biopesticides and provide guidelines for future research.

Schlagwörter:

dsRNA-expressing transgenic plants; nuclear transformation; Pest Control; plastid transformation; RNAi-based biopesticides

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Li, Zhiqian; Zeng, Baosheng; Ling, Lin; Xu, Jun; You, Lang; Aslam, Abu F. M. et al. (2015):

Enhancement of larval RNAi efficiency by over-expressing Argonaute2 in Bombyx mori.

In: *International journal of biological sciences* 11 (2), S. 176–185. DOI: 10.7150/ijbs.10235.

Abstract:

RNA interference has been described as a powerful genetic tool for gene functional analysis and a promising approach for pest management. However, RNAi efficiency varies significantly among insect species due to distinct RNAi machineries. Lepidopteran insects include a large number of pests as well as model insects, such as the silkworm, Bombyx mori. However, only limited success of in vivo RNAi has been reported in lepidoptera, particularly during the larval stages when the worms feed the most and do the most harm to the host plant. Enhancing the efficiency of larval RNAi in lepidoptera is urgently needed to develop RNAi-based pest management strategies. In the present study, we investigate the function of the conserved RNAi core factor, Argonaute2 (Ago2), in mediating B. mori RNAi efficiency. We demonstrate that introducing BmAgo2 dsRNA inhibits the RNAi response in both BmN cells and embryos. Furthermore, we establish several transgenic silkworm lines to assess the

roles of BmAgo2 in larval RNAi. Over-expressing BmAgo2 significantly facilitated both dsRNA-mediated larval RNAi when targeting DsRed using dsRNA injection and shRNA-mediated larval RNAi when targeting BmBlos2 using transgenic shRNA expression. Our results show that BmAgo2 is involved in RNAi in B. mori and provides a promising approach for improving larval RNAi efficiency in B. mori and in lepidopteran insects in general.

Schlagwörter:

Animals; Animals, Genetically Modified/genetics/metabolism; Argonaute Proteins/genetics/metabolism; Bombyx/genetics/metabolism; Larva/genetics/metabolism; RNA Interference; RNA, Double-Stranded/genetics; RNA, Small Interfering/genetics

Kategorien:

1.6 not applicable; 2.2.20 Bombyx mori (Silkworm); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.3 not applicable; 6.5.2 RNAi machinery; 7.1 Concept development

Zeitschriftenaufsatz

List, Fabian; Tarone, Aaron M.; Zhu-Salzman, Keyan; Vargo, Edward L. (2022):

RNA meets toxicology: efficacy indicators from the experimental design of RNAi studies for insect pest management.

In: *Pest management science* 78 (8), S. 3215–3225. DOI: 10.1002/ps.6884.

Abstract:

RNA interference (RNAi) selectively targets genes and silences their expression in vivo, causing developmental defects, mortality and altered behavior. Consequently, RNAi has emerged as a promising research area for insect pest management. However, it is not yet a viable alternative over conventional pesticides despite several theoretical advantages in safety and specificity. As a first step toward a more standardized approach, a machine learning algorithm was used to identify factors that predict trial efficacy. Current research on RNAi for pest management is highly variable and relatively unstandardized. The applied random forest model was able to reliably predict mortality ranges based on bioassay parameters with 72.6% accuracy. Response time and target gene were the most important variables in the model, followed by applied dose, double-stranded RNA (dsRNA) construct size and target species, further supported by generalized linear mixed effect modeling. Our results identified informative trends, supporting the idea that basic principles of toxicology apply to RNAi bioassays and provide initial guidelines standardizing future research similar to studies of traditional insecticides. We advocate for training that integrates genetic, organismal, and toxicological approaches to accelerate the development of RNAi as an effective tool for pest management. © 2022 The Authors. Pest Management Science published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

Schlagwörter:

Animals; Insect Control/methods; Insect Proteins/genetics; Insecta/genetics/metabolism; Research Design; RNA Interference; RNA, Double-Stranded/genetics

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Liu, Shaoshuai; Geng, Shuaifeng; Li, Aili; Mao, Yingbo; Mao, Long (2021):

RNAi technology for plant protection and its application in wheat.

In: *aBIOTECH* 2 (4), S. 365–374. DOI: 10.1007/s42994-021-00036-3.

Abstract:

The RNAi technology takes advantage of the intrinsic RNA interference (RNAi) mechanism that exists in nearly all eukaryotes in which target mRNAs are degraded or functionally suppressed. Significant progress has been made in recent years where RNAi technology is applied to several crops and economic plants for protection against diseases like fungi, pests, and nematode. RNAi technology is also applied in controlling pathogen damages in wheat, one of the most important crops in the world. In this review, we first give a brief introduction of the RNAi technology and the underneath mechanism. We then review the recent progress of its utilization in crops, particular wheat. Finally, we discuss the existing challenges and prospect future development of this technology in crop protection.

Schlagwörter:

dsRNA; Nematoda; pathogen; pests; RNAi (RNA interference); small RNA; wheat (Triticum aestivum)

Kategorien:

1.1.3 Triticum aestivum (wheat); 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Liu, Shaoshuai; Jaouannet, Maelle; Dempsey, D'Maris Amick; Imani, Jafargholi; Coustau, Christine; Kogel, Karl-Heinz (2020):

RNA-based technologies for insect control in plant production.

In: *Biotechnology advances* 39, S. 107463. DOI: 10.1016/j.biotechadv.2019.107463.

Abstract:

RNA interference (RNAi) is a biological process in which small RNA (sRNA) molecules sequence-specifically silence gene expression at the transcriptional or post-transcriptional level, either by directing inhibitory chromatin modifications or by decreasing the stability or translation potential of the targeted mRNA. The trigger for gene silencing is double-stranded RNA (dsRNA) generated from

an endogenous genomic locus or a foreign source, such as a transgene or virus. The process of gene silencing can be exploited in agriculture to control plant diseases and pests. Of the pests that impact crop yield (including nematodes, arthropods, rodents, snails, slugs and birds), insects constitute the largest and most diverse group. Here, we review the "pros" and "cons" of using RNAi technology mediated by dsRNA-expressing transgenic plants (host-induced gene silencing, HIGS) or direct application of chemically synthesized dsRNA to control plant-damaging insects. Rapid progress in elucidating RNAi mechanisms has led to the first commercial products on the market. Given the high potential of RNAi strategies, their use in agriculture, horticulture, and forestry will likely be extensive in the future. However, further studies are needed to improve the efficacy of RNAi-based plant protection strategies and to assess their associated safety risks.

Schlagwörter:

Animals; dsRNA; HIGS (host-induced gene silencing); immunity; insect control; Insecta; insects; pesticide biosafety; plant protection; Plants, Genetically Modified; RNA immune stimulatory activity; RNA Interference; RNA, Double-Stranded; RNAi (RNA interference); small RNA; transgenic plants

Kategorien:

1.6 not applicable; 2.2 insects; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Liu, Shu Hua; Wang, Ai Ying; Yang, Bao Jun; Luo, Ju; Tang, Jian (2017):

Knockdown of an ABC transporter leads to bright red eyes in the brown planthopper, Nilaparvata lugens (Stål) (Hemiptera: Delphacidae).

In: *Journal of Asia-Pacific Entomology* 20 (2), S. 421–428.

Abstract:

Compound eye color is an important biological character of insects, which is determined by the nature of eye pigments. Ommochrome is the solely source of eye color for some insects, while pteridines is also needed for the other insect species. However, little is known about the eye pigment composition for any planthopper. Scarlet is an ABC (ATP-binding cassette) transporter protein, which functions as the transmembrane transporter for ommochrome precursor. The failure of Scarlet function can cause bright red or white eyes in different species, which depending on the nature of eye pigments. Here, we identified a scarlet ortholog gene (Nlst) from the brown planthopper (BPH), Nilaparvata lugens, which is a destructive insect pest of rice. Nlst is the first characterized eye pigment transporter gene from Hemipteran. NlSt, the protein deduced from Nlst, has an open reading frame (ORF) of 629 amino acids with the two sequence logos for eye pigment transporters. Expression profile revealed that Nlst expressed at all development stages and had the highest transcript level in head. Knockdown the Nlst transcript, the wild-type eye color partially changed to bright red, while not to white. Meantime, the ommochrome level in heads reduced to 73.4%. These results suggested that the eye coloration of BPH needs both ommochrome and pteridines pigments. Because nymphal RNAi with Nlst leading to a clearly distinguished phenotype from the control individuals, Nlst maybe a suitable genetic marker to exploit embryonic RNAi technique in this insect pest. (C) 2017 Published by Elsevier B.V. on behalf of Korean Society of Applied Entomology, Taiwan Entomological Society and Malaysian Plant Protection Society.

Schlagwörter:

eye color; Nilaparvata lugens; ommochrome; RNAi (RNA interference); scarlet

Kategorien:

1.1.4 Oryza sativa (rice); 2.2.14 Nilaparvata lugens (Brown planthopper); 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.2.2 eye pigmentation; 7.1 Concept development

Zeitschriftenaufsatz

Lu, Jia-Bao; Wang, Sai-Nan; Ren, Peng-Peng; He, Fang; Li, Qiao; Chen, Jian-Ping et al. (2023):

RNAi-mediated silencing of an egg-specific gene Nllet1 results in hatch failure in the brown planthopper.

In: *Pest management science* 79 (1), S. 415–427. DOI: 10.1002/ps.7210.

Abstract:

BACKGROUND

The brown planthopper (BPH) is one of the most destructive agricultural pests in Asia. RNA interference (RNAi)-mediated pest management has been under development for years, and the selection of appropriate target genes is important for pest-targeted RNAi. C-type lectins (CTLs) are a class of genes that perform a variety of functions, such as the regulation of growth and development.

RESULTS

A CTL-S protein named Nllet1, containing a single calcium ion (Ca2+)-dependent carbohydrate-binding domain (CRD) with a conserved triplet motif QPD was identified and functionally characterized in BPH. Expression profiles at both the transcriptional and translational levels show that Nllet1 accumulates during the serosal cuticle (SC) formation period. Immunofluorescence and immunogold labeling further demonstrated that Nllet1 is located in the serosal endocuticle (en-SC). Maternal RNAi-mediated silencing of Nllet1 disrupted the SC structure, accompanied by a loss of the outward barrier and 100% embryo mortality. Injection of 10 ng dsNllet1 or dsNllet1' per female adult BPH resulted in a total failure of egg hatching.

CONCLUSION

Nllet1 is essential for SC formation and embryonic development in BPH, which helps us understand the important roles of CTL-Ss. Additionally, BPH eggs show high sensitivity to the depletion of Nllet1. This study indicates that Nllet1 is a promising candidate gene that can be used to develop RNAi-based control strategies at the BPH egg stage, and it can also be used as a target for developing novel ovicides.

Schlagwörter:

Asia; Female; Humans; RNA Interference

Kategorien:

1.1.4 Oryza sativa (rice); 2.2.5 Laodelphax striatellus (Brown planthopper); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2.9 microinjection; 6.2.5 embryonic development; 7.1 Concept development

Zeitschriftenaufsatz

Lü, Jing; Guo, Wei; Chen, Shimin; Guo, Mujuan; Qiu, Baoli; Yang, Chunxiao et al. (2020):

Double-stranded RNAs targeting HvRPS18 and HvRPL13 reveal potential targets for pest management of the 28-spotted ladybeetle, Henosepilachna vigintioctopunctata.

In: *Pest management science* 76 (8), S. 2663–2673. DOI: 10.1002/ps.5809.

Abstract:

BACKGROUND

RNA interference (RNAi) is a potential tool for plant protection against insect pests. The great challenge for effective pest control using RNAi in the field is the development of efficient and reliable methods for the production and delivery of double-stranded RNA (dsRNA).

RESULTS

In the present study, we investigated the potential of feeding in vitro synthesized or bacterially expressed dsRNA to populations of the 28-spotted ladybeetle Henosepilachna vigintioctopunctata as a method of biological pest control. Ingestion of in vitro synthesized dsHvRPS18 or dsHvRPL13 led to significant down-regulation of the ribosomal protein-encoding genes HvRPS18 and HvRPL13, respectively, and significantly decreased the survival of H. vigintioctopunctata. Such silencing of HvRPS18 or HvRPL13 expression appeared to be partially dose-dependent and also inhibited the growth of H. vigintioctopunctata and significantly suppressed the expression of digestive enzyme-related genes. Finally, ingestion of bacterially expressed dsHvRPS18 or dsHvRPL13 induced significant mortality in the first and third instars, and in adults.

CONCLUSION

The effectiveness of RNAi-based gene silencing in H. vigintioctopunctata provides a powerful reverse genetic tool for the functional annotation of its genes. This study demonstrates that HvRPS18 and HvRPL13 represent candidate genes for RNAi-based biological control of H. vigintioctopunctata.

Schlagwörter:

Animals; Coleoptera; gene silencing; Pest Control, Biological; RNA Interference; RNA, Double-Stranded

Kategorien:

1.6 not applicable; 2.2.26 Henosepilachna vigintioctopunctata (28-spotted ladybeetle); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2 exogenous; 5.2.3 bmRNAi; 6.1.5 translation; 7.1 Concept development

Zeitschriftenaufsatz

Lü, Jing; Liu, Zhuoqi; Guo, Wei; Guo, Mujuan; Chen, Shimin; Li, Huali et al. (2019):

Feeding Delivery of dsHvSnf7 Is a Promising Method for Management of the Pest Henosepilachnavigintioctopunctata (Coleoptera: Coccinellidae).

In: *Insects* 11 (1). DOI: 10.3390/insects11010034.

Abstract:

RNA interference (RNAi) techniques have emerged as powerful tools in the development of novel management strategies for the control of insect pests, such as Henosepilachna vigintioctopunctata, which is a major solanaceous pest in Asia. Our results showed that levels of HvSnf7 expression were greater in larval midguts than in other tissues. Silencing of HvSnf7 led to greater H. vigintioctopunctata mortality rates and appeared to be time- and partially dose-dependent. Bacterially expressed dsHvSnf7 that was applied to detached plant leaves caused 98, 88, and 60% mortality in 1st and 3rd instars, and adults after 10, 12, and 14 d, respectively; when applied to living plants, bacterially expressed dsHvSnf7 led to mortality in 1st and 3rd instars, with no effect on adults. Bacterially expressed dsHvSnf7 led to improved plant protection against H. vigintioctopunctata. Ultrastructural changes caused by HvSnf7-RNAi in larval midguts showed extensive loss of cellular contents that indicate loss of membrane integrity. This study indicate that HvSnf7 potentially can be used as RNAi target gene for controlling of H. vigintioctopunctata.

Schlagwörter:

feeding RNAi; Henosepilachna vigintiopunctata; HvSnf7; mortality; ultrastructural change

Kategorien:

1.4.9 Solanum melongena (eggplant); 2.2.26 Henosepilachna vigintioctopunctata (28-spotted ladybeetle); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 5.2 exogenous; 5.2.3 bmRNAi; 6.1.15 intracellular transport; 7.2 Concept demonstration

Zeitschriftenaufsatz

Lü, Jing; Liu, Zhuo-Qi; Guo, Wei; Guo, Mu-Juan; Chen, Shi-Min; Yang, Chun-Xiao et al. (2021):

Oral delivery of dsHvlwr is a feasible method for managing the pest Henosepilachna vigintioctopunctata (Coleoptera: Coccinellidae).

In: *Insect science* 28 (2), S. 509–520. DOI: 10.1111/1744-7917.12784.

Abstract:

RNA interference (RNAi) techniques have emerged as powerful tools that facilitate development of novel management strategies for insect pests, such as Henosepilachna vigintioctopunctata (Coleoptera: Coccinellidae), which is a major pest of solanaceous plants in Asia. In this study, the potential of oral delivery of in vitro-synthesized and bacterially expressed double-stranded H.

vigintioctopunctata lesswright (lwr) gene (dsHvlwr) to manage of H. vigintioctopunctata was investigated. Our results showed that the gene Hvlwr had a 480-bp open reading frame and encoded a 160-amino acid protein. Hvlwr expression levels were greater in the fat body than other tissue types. Hvlwr silencing led to greater H. vigintioctopunctata mortality rates and appeared to be time- and partially dose-dependent, likely as a result of the number of hemocytes increasing with dsRNA concentration, but decreasing with time. Bacterially expressed dsHvlwr that was applied to leaf discs caused 88%, 66%, and 36% mortality in 1st instars, 3rd instars, and adults after 10, 10, and 14 d, respectively; when applied to living plants, there was greater mortality in 1st and 3rd instars, but there was no effect on adults. Furthermore, dsHvlwr led to improved plant protection against H. vigintioctopunctata. Our study shows an effective dietary RNAi response in H. vigintioctopunctata and that Hvlwr is a promising RNAi target gene for control of this pest species.

Schlagwörter:

Animals; Coleoptera/genetics/growth & development/physiology; Insect Control/methods; Insect Proteins/genetics/metabolism; Larva/growth & development/physiology; Pupa/growth & development/physiology; RNA Interference

Kategorien:

1.4.9 Solanum melongena (eggplant); 2.2.26 Henosepilachna vigintioctopunctata (28-spotted ladybeetle); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2.3 bmRNAi; 6.1.4 meiosis; 6.5 immune system; 7.2 Concept demonstration

Zeitschriftenaufsatz

Luo, Jing; Liang, Sijia; Li, Jianying; Xu, Zhongping; Li, Lun; Zhu, Bangqin et al. (2017):

A transgenic strategy for controlling plant bugs (Adelphocoris suturalis) through expression of double-stranded RNA homologous to fatty acyl-coenzyme A reductase in cotton.

In: *The New phytologist* 215 (3), S. 1173–1185. DOI: 10.1111/nph.14636.

Abstract:

Plant bugs (Miridae species), which are sap-sucking insects, have emerged as major pests of cotton in China. Most Miridae species are not sensitive to commercial Bacillus thuringiensis (Bt) cotton, resulting in significant economic losses and an increased application of insecticide, which eventually may compromise the future of Bt cotton. We demonstrate that FATTY ACYL-COA REDUCTASE (AsFAR) plays an essential role in the reproduction of the bug Adelphocoris suturalis. Down-regulation of AsFAR expression by injection of double-stranded RNA suppresses ovarian development and female fertility, resulting in females producing few viable offspring. To determine the viability of an RNA interference approach to limit FAR expression and reproductive ability in A. suturalis, a dsRNA targeting the AsFAR gene (dsAsFAR) of A. suturalis was expressed in transgenic cotton plants. AsFAR transcription levels were significantly downregulated in A. suturalis feeding on the transgenic plants. In contained field trials, the transgenic cotton lines significantly suppressed the development of A. suturalis populations and were resistant to damage caused by plant bug infestation. These results suggest a new strategy for the management of plant bug pests of cotton.

Schlagwörter:

Aldehyde Oxidoreductases/chemistry/genetics/metabolism; Amino Acid Sequence; Animals; disease resistance; Down-Regulation; Female; Fertility; Gossypium/genetics/parasitology; Heteroptera/enzymology; Ovary/enzymology; Pest Control, Biological; phenotype; Plants, Genetically Modified; RNA, Double-Stranded/metabolism; Transcription, Genetic; Transformation, Genetic

Kategorien:

1.2.2 Gossypium hirsutum (cotton); 2.2.34 Adelphocoris suturalis (Miridae); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.1 endogenous; 5.1.1 stable transgene; 6.3 energy metabolism; 7.2 Concept demonstration

Zeitschriftenaufsatz

Ma, Weihua; Zhao, Xianxin; Yin, Chuanlin; Jiang, Fan; Du, Xiaoyong; Chen, Taiyu et al. (2020):

A chromosome-level genome assembly reveals the genetic basis of cold tolerance in a notorious rice insect pest, Chilo suppressalis.

In: *Molecular ecology resources* 20 (1), S. 268–282. DOI: 10.1111/1755-0998.13078.

Abstract:

The rice stem borer, Chilo suppressalis, is one of the most damaging insect pests to rice production worldwide. Although C. suppressalis has been the focus of numerous studies examining cold tolerance and diapause, plant-insect interactions, pesticide targets and resistance, and the development of RNAi-mediated pest management, the absence of a high-quality genome has limited deeper insights. To address this limitation, we generated a draft C. suppressalis genome constructed from both Illumina and PacBio sequences. The assembled genome size was 824.35 Mb with a contig N50 of 307 kb and a scaffold N50 of 1.75 Mb. Hi-C scaffolding assigned 99.2% of the bases to one of 29 chromosomes. Based on universal single-copy orthologues (BUSCO), the draft genome assembly was estimated to be 97% complete and is predicted to encompass 15,653 protein-coding genes. Cold tolerance is an extreme survival strategy found in animals. However, little is known regarding the genetic basis of the winter ecology of C. suppressalis. Here, we focused our orthologous analysis on those gene families associated with animal cold tolerance. Our finding provided the first genomic evidence revealing specific cold-tolerant strategies in C. suppressalis, including those involved in glucose-originated glycerol biosynthesis, triacylglycerol-originated glycerol biosynthesis, fatty acid synthesis and trehalose transport-intermediate cold tolerance. The high-quality C. suppressalis genome provides a valuable resource for research into a broad range of areas in molecular ecology, and subsequently benefits developing modern pest control strategies.

Schlagwörter:

Animals; Chromosomes, Insect/genetics; Genome Size; Genome, Insect; Moths/classification/genetics; Oryza/parasitology; Phylogeny; Plant Diseases/parasitology

Kategorien:

1.1.4 Oryza sativa (rice); 2.2.9 Chilo suppressalis (Rice stem borer); 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.1 Concept development

Zeitschriftenaufsatz

Ma, Zhong-Zheng; Zhou, Hang; Wei, Yan-Long; Yan, Shuo; Shen, Jie (2020):

A novel plasmid-Escherichia coli system produces large batch dsRNAs for insect gene silencing.

In: *Pest management science* 76 (7), S. 2505–2512. DOI: 10.1002/ps.5792.

Abstract:

BACKGROUND

RNA interference (RNAi)-based pest management requires efficient delivery and large-batch production of double-stranded (ds)RNA. We previously developed a nanocarrier-mediated dsRNA delivery system that could penetrate an insect's body and efficiently silence gene expression. However, there is a great need to improve the plasmid-Escherichia coli system for the mass production of dsRNA. Here, for efficient dsRNA production, we removed the rnc gene encoding endoribonuclease RNase III in E. coli BL21(DE3) and matched with the RNAi expression vector containing a single T7 promoter.

RESULTS

The novel pET28-BL21(DE3) RNase III-system was successfully constructed to express vestigial (vg)-dsRNA against Harmonia axyridis. dsRNA was extracted and purified from cell cultures in four E. coil systems, and the yields of dsRNA in pET28-BL21(DE3) RNase III-, pET28-HT115(DE3), L4440-BL21(DE3) RNase III- and L4440-HT115(DE3) were 4.23, 2.75, 0.88 and 1.30 μg mL-1 respectively. The dsRNA expression efficiency of our novel E. coil system was three times that of L4440-HT115(DE3), a widely used dsRNA production system. The RNAi efficiency of dsRNA produced by our system and by biochemical synthesis was comparable when injected into Harmonia axyridis.

CONCLUSION

Our system expressed dsRNA more efficiently than the widely used L4440-HT115(DE3) system, and the produced dsRNA showed a high gene-silencing effect. Notably, our pET28-BL21(DE3) RNase III-system provides a novel method for the mass production of dsRNA at low cost and high efficiency, which may promote gene function analysis and RNAi-based pest management.

Schlagwörter:

Animals; Escherichia coli; Insecta; Plasmids; RNA Interference; RNA, Double-Stranded

Kategorien:

1.6 not applicable; 2.2 insects; 2.2.8 Harmonia axyridis (Asian ladybug); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2 exogenous; 5.2.9 microinjection; 6.9 not applicable; 7.1 Concept development

Zeitschriftenaufsatz

Machado, Ana Karla; Brown, Neil A.; Urban, Martin; Kanyuka, Kostya; Hammond-Kosack, Kim E. (2018):

RNAi as an emerging approach to control Fusarium head blight disease and mycotoxin contamination in cereals.

In: *Pest management science* 74 (4), S. 790–799. DOI: 10.1002/ps.4748.

Abstract:

Fusarium graminearum is a major fungal pathogen of cereals worldwide, causing seedling, stem base and floral diseases, including Fusarium head blight (FHB). In addition to yield and quality losses, FHB contaminates cereal grain with mycotoxins, including deoxynivalenol, which are harmful to human, animal and ecosystem health. Currently, FHB control is only partially effective due to several intractable problems. RNA interference (RNAi) is a natural mechanism that regulates gene expression. RNAi has been exploited in the development of new genomic tools that allow the targeted silencing of genes of interest in many eukaryotes. Host-induced gene silencing (HIGS) is a transgenic technology used to silence fungal genes in planta during attempted infection and thereby reduces disease levels. HIGS relies on the host plant's ability to produce mobile small interfering RNA molecules, generated from long double-stranded RNA, which are complementary to targeted fungal genes. These molecules are transferred from the plant to invading fungi via an uncharacterised mechanism, to cause gene silencing. Here, we describe recent advances in RNAi-mediated control of plant pathogenic fungi, highlighting the key advantages and disadvantages. We then discuss the developments and implications of combining HIGS with other methods of disease control. © 2017 The Authors. Pest Management Science published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

Schlagwörter:

Edible Grain/microbiology; Fusarium/genetics; Genes, Fungal/genetics; Mycotoxins/analysis; Plant Diseases/microbiology/prevention & control; RNA Interference

Kategorien:

1.6 not applicable; 2.1.1 Fusarium graminearum; 3.3 not applicable; 4.3 not applicable; 5.1 endogenous; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Mai, Jiantao; Liao, Lingling; Ling, Rongsong; Guo, Xiaolong; Lin, Jingying; Mo, Beixin et al. (2021):

Study on RNAi-based herbicide for Mikania micrantha.

In: *Synthetic and systems biotechnology* 6 (4), S. 437–445. DOI: 10.1016/j.synbio.2021.11.005.

Abstract:

The invasive plant Mikania micrantha Kunth (M. micrantha) from South America poses a significant threat to the stability and biodiversity of ecosystems. However, an effective and economical method to control M. micrantha is still lacking. RNA interference (RNAi) has been widely studied and applied in agriculture for trait improvement. Spray-induced gene silencing (SIGS) can produce RNAi silencing effects without introducing heritable modifications to the plant genome and is becoming a novel nontransformation strategy for plant protection. In this study, the genes encoding chlorophyll a/b-binding proteins were selected as targets of RNAi, based on high-throughput sequencing of M. micrantha transcriptome and bioinformatic analyses of sequence specificity. Three types of RNAi molecules, double-stranded RNA, RNAi nanomicrosphere, and short hairpin RNA (shRNA), with their corresponding short interfering RNA sequences were designed and synthesized for SIGS vector construction, from which each RNAi molecule was transcribed and extracted to be sprayed on M. micrantha leaves. Whereas water-treated control leaves remained green, leaves treated with RNAi molecules turned yellow and eventually wilted. Quantitative real-time PCR showed that the expression levels of target genes were significantly reduced in the RNAi-treated groups compared with those of the control, suggesting that all three types of RNAi herbicides effectively silenced the endogenous target genes, which are essential for the growth of M. micrantha. We also found that shRNA showed better silencing efficiency than the other two molecules. Taken together, our study successfully designed three types of RNAi-based herbicides that specifically silenced endogenous target genes and controlled the growth of M. micrantha. Moreover, we identified a gene family encoding chlorophyll a/b-binding proteins that is important for the growth and development of M. micrantha and could serve as potential targets for controlling the spread of M. micrantha.

Schlagwörter:

chlorophyll a/b-binding protein; invasive plant; Mikania micrantha; nucleic acid bioherbicide; RNAi (RNA interference); SIGS (spray-induced gene silencing)

Kategorien:

1.6 not applicable; 2.3.1 Mikania micrantha; 3.3 not applicable; 4.1.1 shRNA; 4.1.1.2 RNAi nanomicrosphere; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2.2 spray (SIGS); 6.3.5 photosynthesis; 7.1 Concept development

Zeitschriftenaufsatz

Maksimov, I. V.; Shein, M. Yu.; Burkhanova, G. F. (2022):

RNA Interference in Plant Protection from Fungal and Oomycete Infection.

In: *Appl Biochem Microbiol* 58 (S1), S16-S31. DOI: 10.1134/S0003683822100106.

Abstract:

Phytopathogenic fungi pose a threat to food security, limiting the biological potential of agricultural crops and reducing the quality of products. New plant protection methods based on natural systemic and cellular phytoimmunity are being developed, where a unique mechanism, described by the term “RNA interference” (RNAi), occupies a special place. RNAi regulates the expression of target genes in a homologically dependent manner; with the involvement of a protein complex designated as RISC (RNA-induced silencing complex), on the one hand, it protects plants from pathogens, but on the other hand, pathogens use it as a virulence factor. Cases of bilateral exchange of small RNAs between plants and pathogens that affect them through extracellular vesicles have been described. This review discusses the role of small RNAs, as well as the DCL, AGO, and RdR proteins, in the infection of plants with pathogenic fungi and oomycetes, and the prospects for using RNAi in the development of environmentally friendly modern plant protection products.

Schlagwörter:

phytoimmunity; RNAi

Kategorien:

1.6 not applicable; 2.1 fungi; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Mann, Christopher W. G.; Sawyer, Anne; Gardiner, Donald M.; Mitter, Neena; Carroll, Bernard J.; Eamens, Andrew L. (2023):

RNA-Based Control of Fungal Pathogens in Plants.

In: *International journal of molecular sciences* 24 (15). DOI: 10.3390/ijms241512391.

Abstract:

Our duty to conserve global natural ecosystems is increasingly in conflict with our need to feed an expanding population. The use of conventional pesticides not only damages the environment and vulnerable biodiversity but can also still fail to prevent crop losses of 20-40% due to pests and

pathogens. There is a growing call for more ecologically sustainable pathogen control measures. RNA-based biopesticides offer an eco-friendly alternative to the use of conventional fungicides for crop protection. The genetic modification (GM) of crops remains controversial in many countries, though expression of transgenes inducing pathogen-specific RNA interference (RNAi) has been proven effective against many agronomically important fungal pathogens. The topical application of pathogen-specific RNAi-inducing sprays is a more responsive, GM-free approach to conventional RNAi transgene-based crop protection. The specific targeting of essential pathogen genes, the development of RNAi-nanoparticle carrier spray formulations, and the possible structural modifications to the RNA molecules themselves are crucial to the success of this novel technology. Here, we outline the current understanding of gene silencing pathways in plants and fungi and summarize the pioneering and recent work exploring RNA-based biopesticides for crop protection against fungal pathogens, with a focus on spray-induced gene silencing (SIGS). Further, we discuss factors that could affect the success of RNA-based control strategies, including RNA uptake, stability, amplification, and movement within and between the plant host and pathogen, as well as the cost and design of RNA pesticides.

Schlagwörter:

Biological Control Agents; Crops, Agricultural/genetics; Ecosystem; Pesticides; Plant Diseases/genetics/prevention & control/microbiology; RNA Interference; RNA, Small Interfering/genetics

Kategorien:

1.6 not applicable; 2.1 fungi; 3.3 not applicable; 4.3 not applicable; 5.2.2 spray (SIGS); 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Mao, Yingbo; Xue, XueYi; Chen, XiaoYa (2009):

Are small RNAs a big help to plants?

In: *Science in China. Series C, Life sciences* 52 (3), S. 212–223. DOI: 10.1007/s11427-009-0034-3.

Abstract:

The discovery of RNA interference (RNAi) has augmented our knowledge of gene regulation and presents a fascinating technology that has a great potential for application in genetic analysis, disease therapy, plant protection, and many other areas. In this review, we will focus on the biological functions of RNAi and its application in agriculture with a brief introduction to the history of its discovery and molecular mechanism.

Schlagwörter:

Crops, Agricultural/genetics; Gene Expression Regulation, Plant; gene silencing; Models, Genetic; Plants, Genetically Modified/genetics; Plants/genetics; RNA Interference; RNA, Small Interfering/genetics; RNA, Untranslated/genetics

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Mcloughlin, Austein G.; Walker, Philip L.; Wytinck, Nick; Sullivan, Daniel S.; Whyard, Steve; Belmonte, Mark F. (2018):

Developing new RNA interference technologies to control fungal pathogens.

In: *Canadian Journal of Plant Pathology* 40 (3), S. 325–335. DOI: 10.1080/07060661.2018.1495268.

Abstract:

Current agricultural output is challenged by considerable losses in crop yield and post-harvest storage due to fungal infection. Traditional chemical fungicides used to treat these fungi can be ineffective and harmful to the environment if not used properly. With fungicide resistance increasing in fungal pathogens, new environmentally friendly and sustainable technologies are required to manage diseases on the world's most important crops. RNA interference (RNAi) is an intrinsic cellular mechanism, mediated by double-stranded RNA (dsRNA), which can suppress protein expression through targeted destruction of mRNAs. With recent advances in dsRNA delivery or expression in plants, this mechanism has the potential to provide alternative disease management strategies. Examples of RNAi-based control to manage pathogenic fungal species are steadily increasing, and the technology offers new options to increase species-specificity and/or potency against fungi for which existing fungicides have been ineffective. RNAi technology can be adapted to provide either robust and multi-crop plant protection using topical sprays or can provide more durable resistance through transgene expression of dsRNAs within susceptible plant tissues. Using RNA sequencing to identify fungal gene targets, RNAi-based control technology continues to show promise as an alternative to traditional agrochemicals for crop protection.

Schlagwörter:

fungal pathogens; green technologies; rna sequencing; RNAi (RNA interference)

Kategorien:

1.6 not applicable; 2.1 fungi; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Mekapogu, Manjulatha; Jung, Jae-A; Kwon, Oh-Keun; Ahn, Myung-Suk; Song, Hyun-Young; Jang, Seonghoe (2021):

Recent Progress in Enhancing Fungal Disease Resistance in Ornamental Plants.

In: *International journal of molecular sciences* 22 (15). DOI: 10.3390/ijms22157956.

Abstract:

Fungal diseases pose a major threat to ornamental plants, with an increasing percentage of pathogen-driven host losses. In ornamental plants, management of the majority of fungal diseases primarily depends upon chemical control methods that are often non-specific. Host basal resistance, which is deficient in many ornamental plants, plays a key role in combating diseases. Despite their economic importance, conventional and molecular breeding approaches in ornamental plants to facilitate disease resistance are lagging, and this is predominantly due to their complex genomes, limited availability of gene pools, and degree of heterozygosity. Although genetic engineering in ornamental plants offers feasible methods to overcome the intrinsic barriers of classical breeding, achievements have mainly been reported only in regard to the modification of floral attributes in ornamentals. The unavailability of transformation protocols and candidate gene resources for several ornamental crops presents an obstacle for tackling the functional studies on disease resistance. Recently, multiomics technologies, in combination with genome editing tools, have provided shortcuts to examine the molecular and genetic regulatory mechanisms underlying fungal disease resistance, ultimately leading to the subsequent advances in the development of novel cultivars with desired fungal disease-resistant traits, in ornamental crops. Although fungal diseases constitute the majority of ornamental plant diseases, a comprehensive overview of this highly important fungal disease resistance seems to be insufficient in the field of ornamental horticulture. Hence, in this review, we highlight the representative mechanisms of the fungal infection-related resistance to pathogens in plants, with a focus on ornamental crops. Recent progress in molecular breeding, genetic engineering strategies, and RNAi technologies, such as HIGS and SIGS for the enhancement of fungal disease resistance in various important ornamental crops, is also described.

Schlagwörter:

Disease Resistance/genetics; Mitosporic Fungi/growth & development; plant breeding; Plant Diseases/genetics/microbiology; Plants, Genetically Modified/genetics/microbiology

Kategorien:

1.5.1 "ornamental plants"; 2.1 fungi; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Mezzetti, Bruno; Smagghe, Guy; Arpaia, Salvatore; Christiaens, Olivier; Dietz-Pfeilstetter, Antje; Jones, Huw et al. (2020):

RNAi: What is its position in agriculture?

In: *J Pest Sci* 93 (4), S. 1125–1130. DOI: 10.1007/s10340-020-01238-2.

Abstract:

RNA interference (RNAi) is being developed and exploited to improve plants by modifying endogenous gene expression as well as to target pest and pathogen genes both within plants (i.e. host-induced gene silencing) and/or as topical applications (e.g. spray-induced gene silencing). RNAi is a natural mechanism which can be exploited to make a major contribution towards integrated pest management and sustainable agricultural strategies needed worldwide to secure current and future food production. RNAi plants are being assessed and regulated using existing regulatory frameworks for GMO. However, there is an urgent need to develop appropriate science-based risk assessment procedures for topical RNAi applications within existing plant protection products legislation.

Schlagwörter:

agriculture; biosafety; dsRNA; HIGS (host-induced gene silencing); regulation; RNAi (RNA interference); SIGS (spray-induced gene silencing)

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Miyata, Keita; Ramaseshadri, Parthasarathy; Zhang, Yuanji; Segers, Gerrit; Bolognesi, Renata; Tomoyasu, Yoshinori (2014):

Establishing an in vivo assay system to identify components involved in environmental RNA interference in the western corn rootworm.

In: *PloS one* 9 (7), e101661. DOI: 10.1371/journal.pone.0101661.

Abstract:

The discovery of environmental RNA interference (RNAi), in which gene expression is suppressed via feeding with double-stranded RNA (dsRNA) molecules, opened the door to the practical application

of RNAi-based techniques in crop pest management. The western corn rootworm (WCR, Diabrotica virgifera virgifera) is one of the most devastating corn pests in North America. Interestingly, WCR displays a robust environmental RNAi response, raising the possibility of applying an RNAi-based pest management strategy to this pest. Understanding the molecular mechanisms involved in the WCR environmental RNAi process will allow for determining the rate limiting steps involved with dsRNA toxicity and potential dsRNA resistance mechanisms in WCR. In this study, we have established a two-step in vivo assay system, which allows us to evaluate the involvement of genes in environmental RNAi in WCR. We show that laccase 2 and ebony, critical cuticle pigmentation/tanning genes, can be used as marker genes in our assay system, with ebony being a more stable marker to monitor RNAi activity. In addition, we optimized the dsRNA dose and length for the assay, and confirmed that this assay system is sensitive to detect well-known RNAi components such as Dicer-2 and Argonaute-2. We also evaluated two WCR sid1- like (sil) genes with this assay system. This system will be useful to quickly survey candidate systemic RNAi genes in WCR, and also will be adaptable for a genome-wide RNAi screening to give us an unbiased view of the environmental/systemic RNAi pathway in WCR.

Schlagwörter:

Animals; Coleoptera/genetics/growth & development; Gene Dosage; Genes, Insect; Insect Control/methods; Life Cycle Stages/genetics; Pest Control, Biological/methods; phenotype; Reproducibility of Results; RNA Interference; RNA, Double-Stranded

Kategorien:

1.6 not applicable; 2.2.2 Diabrotica virgifera virgifera (Western corn rootworm); 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.1 Concept development

Zeitschriftenaufsatz

Mosa, Mohamed A.; Youssef, Khamis (2021):

Topical delivery of host induced RNAi silencing by layered double hydroxide nanosheets: An efficient tool to decipher pathogenicity gene function of Fusarium crown and root rot in tomato.

In: *Physiological and Molecular Plant Pathology* 115, S. 101684.

Abstract:

RNAi technique was used to regulate Fusarium crown and root rot in tomatoes caused by F. oxysporum f. sp. radicis-lycopersici (FORL), by targeting three essential genes namely FoCYP51, FoChs1, and FoEF2, considering the fungicide site of action. The designed naked dsRNA was delivered on a hexagonal mono-dispersed and biodegradable layered double hydroxide (LDH) nanosheets with 30-90 nm diameter, providing high protection and stability for dsRNA with long term sustained release manner inside plant cells. The laboratory findings revealed the functional role of the three selected genes in reducing FORL's invasive growth on tomato fruits, indicating their involvement in FORL pathogenesis on tomatoes, and could be good targets for disease control using RNAi. Three different practical methods were applied for topical delivery of dsRNA into the plant cells: i) leaves spray; ii) petiole adsorption; and iii) root dipping, demonstrating three significant levels of spray-induced gene silencing (SIGS) efficacy in controlling FORL virulence infecting tomato plants. Significantly, topical spray of dsRNA delivered on LDH nanosheets provided Fusarium crown and root

rot protection for at least 60 days. Obtained findings introduce critical information data on the potential to exploit spray-induced gene silencing (SIGS) as an effective alternative sustainable strategy for plant disease management. Given the ease design and production of biodegradable nano-delivery systems with high specificity and sustainability, and ease of applicability against many disease caused fungi. The use of target-specific dsRNA as an anti-fungal agent has a lot of promise and provide exceptional potential as a unique disease control and plant protection strategy.

Schlagwörter:

Fusarium crown and root rot; LDH nanosheets; post-transcriptpional gene silencing (PTGS)

Kategorien:

1.4.8 Solanum lycopersium (tomato); 2.1.10 Fusarium oxysporum; 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2.2 spray (SIGS); 5.2.4.1 nanoparticle-mediated dsRNA; 5.2.5 petiole adsorption; 5.2.6 root dipping; 6.1.11 ergosterol biosynthesis; 7.2 Concept demonstration

Patentschrift

Narva, Kenneth; Sridharan, Krishnakumar; Devisetty, Upendra; Barros, Rodrigues Thais; Oppert, Cris; Desau, Suresh et al. (2021):

RNA-BASED CONTROL OF LEPIDOPTERAN PESTS.

Angemeldet durch GREENLIGHT BIOSCIENCES INC [US] am 13.05.2021. Anmeldenr: WO2021US32334 20210513. Veröffentlichungsnr: WO2021231791 (A2). A01N57/16. Prioriätsdaten: US202063024133P 20200513;US202063106614P 20201028.

Abstract:

Disclosed herein are compositions and methods of controlling pests that involves administering RNA interfering agents. Particularly exemplified is the control of Lepidopteran species such as diamondback moths and fall armyworms. Also disclosed is the use of double-stranded RNA molecules to target certain genes in Lepidopteran species.

L'invention concerne des compositions et des procédés de lutte contre les organismes nuisibles qui consistent à administrer des agents d'interférence à ARN. En particulier, l'invention concerne la lutte contre des espèces de lépidoptères, telles que les fausses-teignes des crucifères et les légionnaires d'automne. L'invention concerne également l'utilisation de molécules d'ARN double brin pour cibler certains gènes dans des espèces de lépidoptères.

Kategorien:

2.2.35 Lepidopteran species; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation

Patentschrift

Narva, Kenneth E.; Fishilevich, Elane; Rangasamy, Murugesan; Frey, Meghan L.; Lo, Wendy; Worden, Sarah E.; Gandra, Premchand (2016):

RAB5 NUCLEIC ACID MOLECULES THAT CONFER RESISTANCE TO COLEOPTERAN AND HEMIPTERAN PESTS.

Angemeldet durch DOW AGROSCIENCES LLC [US] am 28.10.2016. Anmeldenr: WO2016US59248 20161028. Veröffentlichungsnr: WO2017079036 (A1). A01N63/50;C12N1/21;C12N15/113. Prioriätsdaten: US201562249463P 20151102.

Abstract:

This disclosed subject matter concerns nucleic acid molecules and methods of use thereof for control of coleopteran pests through RNA interference-mediated inhibition of target coding and transcribed non-coding sequences in coleopteran pests. The disclosure also concerns methods for making transgenic plants that express nucleic acid molecules useful for the control of coleopteran pests, and the plant cells and plants obtained thereby.

Cette invention concerne des molécules d'acide nucléique et des procédés d'utilisation de celles-ci dans la lutte contre les coléoptères nuisibles par l'intermédiaire d'une inhibition à médiation par interférence ARN de séquences codantes cibles et de séquences non codantes transcrites chez les coléoptères nuisibles. L'invention concerne également des procédés de production de plantes transgéniques qui expriment des molécules d'acide nucléique utiles pour la lutte contre les coléoptères nuisibles, ainsi que les cellules végétales et les plantes ainsi obtenues.

Kategorien:

2.2.32 Hemiptera; 2.2.36 Coleoptera

Patentschrift

Narva, Kenneth E.; Li, Huarong; Geng, Chaoxian; Larrinua, Ignacio Mario; Elango, Navin; Woosley, Aaron T. et al. (2016):

NUCLEIC ACID MOLECULES THAT CONFER RESISTANCE TO COLEOPTERAN PESTS.

Angemeldet durch DOW AGROSCIENCES LLC [US] am 20.10.2016. Anmeldenr: CA20163003429 20161020. Veröffentlichungsnr: CA3003429 (A1). A01H5/00;C07K14/325;C12N15/113;C12N15/63;C12N15/66;C12N15/67;C12N15/82;C12N15/87. Prioriätsdaten: US201562247391P 20151028;WO2016US57848 20161020.

Abstract:

This disclosure concerns nucleic acid molecules and methods of use thereof for control of coleopteran pests through RNA interference-mediated inhibition of target coding and transcribed non-coding sequences in coleopteran pests. The disclosure also concerns methods for making transgenic plants that express nucleic acid molecules useful for the control of coleopteran pests, and the plant cells and plants obtained thereby.

Kategorien:

2.2.36 Coleoptera; 5.1 endogenous; 5.1.1 stable transgene

Zeitschriftenaufsatz

Niehl, Annette; Soininen, Marjukka; Poranen, Minna M.; Heinlein, Manfred (2018):

Synthetic biology approach for plant protection using dsRNA.

In: *Plant Biotechnol J* 16 (9), S. 1679–1687. DOI: 10.1111/pbi.12904.

Abstract:

Pathogens induce severe damages on cultivated plants and represent a serious threat to global food security. Emerging strategies for crop protection involve the external treatment of plants with double-stranded (ds)RNA to trigger RNA interference. However, applying this technology in greenhouses and fields depends on dsRNA quality, stability and efficient large-scale production. Using components of the bacteriophage phi6, we engineered a stable and accurate in vivo dsRNA production system in Pseudomonas syringae bacteria. Unlike other in vitro or in vivo dsRNA production systems that rely on DNA transcription and postsynthetic alignment of single-stranded RNA molecules, the phi6 system is based on the replication of dsRNA by an RNA-dependent RNA polymerase, thus allowing production of high-quality, long dsRNA molecules. The phi6 replication complex was reprogrammed to multiply dsRNA sequences homologous to tobacco mosaic virus (TMV) by replacing the coding regions within two of the three phi6 genome segments with TMV sequences and introduction of these constructs into P. syringae together with the third phi6 segment, which encodes the components of the phi6 replication complex. The stable production of TMV dsRNA was achieved by combining all the three phi6 genome segments and by maintaining the natural dsRNA sizes and sequence elements required for efficient replication and packaging of the segments. The produced TMV-derived dsRNAs inhibited TMV propagation when applied to infected Nicotiana benthamiana plants. The established dsRNA production system enables the broad application of dsRNA molecules as an efficient, highly flexible, nontransgenic and environmentally friendly approach for protecting crops against viruses and other pathogens.

Schlagwörter:

dsRNA production technology; RNAi (RNA interference); sustainable crop protection

Kategorien:

1.5.4 Nicotiana benthamiana; 2.4.1 Tobacco mosaic virus; 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2.3 bmRNAi; 7.2 Concept demonstration

Zeitschriftenaufsatz

Niño-Sánchez, Jonatan; Chen, Li-Hung; Souza, Jorge Teodoro de; Mosquera, Sandra; Stergiopoulos, Ioannis (2021):

Targeted Delivery of Gene Silencing in Fungi Using Genetically Engineered Bacteria.

In: *Journal of fungi (Basel, Switzerland)* 7 (2). DOI: 10.3390/jof7020125.

Abstract:

Exploiting RNA interference (RNAi) in disease control through non-transformative methods that overcome the hurdle of producing transgenic plants has attracted much attention over the last years. Here, we explored such a method and used non-pathogenic bacteria as a versatile system for delivering RNAi to fungi. Specifically, the RNaseIII-null mutant strain of Escherichia coli HT115(DE3) was transformed with two plasmid vectors that enabled the constitutive or IPTG-inducible production of double-stranded RNAs (dsRNAs) against genes involved in aflatoxins production in Aspergillus flavus (AflC) or virulence of Botrytis cinerea (BcSAS1). To facilitate the release of the dsRNAs, the bacterial cells were further genetically engineered to undergo a bacteriophage endolysin R-mediated autolysis, following a freeze-thaw cycle. Exposure under in vitro conditions of A. flavus or B. cinerea to living bacteria or their whole-cell autolysates induced silencing of AflC and BcSAS1 in a bacteria concentration-dependent manner, and instigated a reduction in aflatoxins production and mycelial growth, respectively. In planta applications of the living bacteria or their crude whole-cell autolysates produced similar results, thus creating a basis for translational research. These results demonstrate that bacteria can produce biologically active dsRNA against target genes in fungi and that bacteria-mediated RNAi can be used to control fungal pathogens.

Schlagwörter:

aflatoxins; Aspergillus flavus; bacterial autolysis; Botrytis cinerea; cross-kingdom RNAi; dsRNA; Escherichia coli HT115 (DE3); HIGS (host-induced gene silencing); RNAi (RNA interference); SIGS (spray-induced gene silencing)

Kategorien:

1.6 not applicable; 2.1.2 Botrytis cinerea; 2.1.12 Aspergillus flavus; 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2.3 bmRNAi; 6.7.5 aflatoxin production; 6.7.8 infection mechanisms (virulence factors); 7.1 Concept development

Zeitschriftenaufsatz

Niño-Sánchez, Jonatan; Sambasivam, Prabhakaran T.; Sawyer, Anne; Hamby, Rachael; Chen, Angela; Czislowski, Elizabeth et al. (2022):

BioClay™ prolongs RNA interference-mediated crop protection against Botrytis cinerea.

In: *Journal of integrative plant biology* 64 (11), S. 2187–2198. DOI: 10.1111/jipb.13353.

Abstract:

One of the most promising tools for the control of fungal plant diseases is spray-induced gene silencing (SIGS). In SIGS, small interfering RNA (siRNA) or double-stranded RNA (dsRNA) targeting essential or virulence-related pathogen genes are exogenously applied to plants and postharvest products to trigger RNA interference (RNAi) of the targeted genes, inhibiting fungal growth and disease. However, SIGS is limited by the unstable nature of RNA under environmental conditions. The use of layered double hydroxide or clay particles as carriers to deliver biologically active dsRNA, a formulation termed BioClay™, can enhance RNA durability on plants, prolonging its activity against pathogens. Here, we demonstrate that dsRNA delivered as BioClay can prolong protection against Botrytis cinerea, a major plant fungal pathogen, on tomato leaves and fruit and on mature chickpea plants. BioClay increased the protection window from 1 to 3 weeks on tomato leaves and from 5 to 10 days on tomato fruits, when compared with naked dsRNA. In flowering chickpea plants, BioClay provided prolonged protection for up to 4 weeks, covering the critical period of poding, whereas naked dsRNA provided limited protection. This research represents a major step forward for the adoption of SIGS as an eco-friendly alternative to traditional fungicides.

Schlagwörter:

Botrytis; crop protection; Plant Diseases/prevention & control/microbiology; Plants/genetics; RNA Interference; RNA, Double-Stranded/genetics; RNA, Small Interfering/genetics; Solanum lycopersicum/genetics

Kategorien:

1.4.7 Cicer aestivum (chickpea); 1.4.8 Solanum lycopersium (tomato); 2.1.2 Botrytis cinerea; 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2.4.5 BioClay-mediated RNAi; 6.1.15 intracellular transport; 6.9 not applicable; 7.2 Concept demonstration

Zeitschriftenaufsatz

Niu, Dongdong; Hamby, Rachael; Sanchez, Jonatan Nino; Cai, Qiang; Yan, Qin; Jin, Hailing (2021):

RNAs - a new frontier in crop protection.

In: *Current opinion in biotechnology* 70, S. 204–212. DOI: 10.1016/j.copbio.2021.06.005.

Abstract:

Small RNA (sRNA)-mediated RNA interference (RNAi) is a regulatory mechanism conserved in almost all eukaryotes. sRNAs play a critical role in host pathogen interactions either endogenously or by traveling between the interacting organisms and inducing 'cross-Kingdom RNAi' in the counterparty. Cross-kingdom RNAi is the mechanistic basis of host-induced gene silencing (HIGS), which relies on genetically expressing pathogen-gene targeting RNAs in crops, and has been successfully utilized against both microbial pathogens and pests. HIGS is limited by the need to produce genetically engineered crops. Recent studies have demonstrated that double-stranded RNAs and sRNAs can be efficiently taken up by many fungal pathogens, and induce gene silencing in fungal cells. This mechanism, termed 'environmental RNAi', allows direct application of pathogen-gene targeting RNAs onto crops to silence fungal virulence-related genes for plant protection. In this review, we will focus on how we can leverage cross-kingdom RNAi and environmental RNAi for crop disease control.

Schlagwörter:

crop protection; Crops, Agricultural/genetics; RNA Interference; RNA, Plant; RNA, Small Interfering

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.2.2 spray (SIGS); 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Niu, Jinzhi; Shen, Guangmao; Christiaens, Olivier; Smagghe, Guy; He, Lin; Wang, Jinjun (2018):

Beyond insects: current status and achievements of RNA interference in mite pests and future perspectives.

In: *Pest management science* 74 (12), S. 2680–2687. DOI: 10.1002/ps.5071.

Abstract:

Mites comprise a group of key agricultural pests on a wide range of crops. They cause harm through feeding on the plant and transferring dangerous pathogens, and the rapid evolution of pesticide resistance in mites highlights the need for novel control methods. Currently, RNA interference (RNAi) shows great potential for insect pest control. Here, we review the literature regarding RNAi in mite pests. We discuss different target genes and RNAi efficiency in various mite species, a promising Varroa control program using RNAi, the synergy of RNAi with plant defense mechanisms and microorganisms, and current understanding of systemic movement of double-stranded RNA (dsRNA). On the basis of this evidence, we can conclude that there is clear potential for application of RNAi-based mite control, but further research on several aspects of RNAi in mites is needed, including: (i) the factors influencing RNAi efficiency, (ii) the mechanism of environmental RNAi and cross-kingdom dsRNA trafficking, (iii) the mechanism of possible systemic and parental RNAi, and (iv) non-target effects, specifically in predatory mites, which should be considered during RNAi target selection. © 2018 Society of Chemical Industry.

Schlagwörter:

Animals; Crops, Agricultural/metabolism; Insect Control/methods; Mites/drug effects/genetics; RNA Interference

Kategorien:

1.6 not applicable; 2.5.2 mites; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Niu, Lin; Yan, Haixia; Sun, Yajie; Zhang, Delin; Ma, Weihua; Lin, Yongjun (2022):

Nanoparticle facilitated stacked-dsRNA improves suppression of the Lepidoperan pest Chilo suppresallis.

In: *Pesticide biochemistry and physiology* 187, S. 105183. DOI: 10.1016/j.pestbp.2022.105183.

Abstract:

In recent years, gene knockdown technology using double-stranded RNA (dsRNA) has been widely used as an environment-friendly pest control strategy, but its instability and limited cellular uptake have limited its overall effect. Studies have shown that the efficiency of single dsRNA can be improved by using various nanomaterials. However, the effect of stacked-dsRNA wrapped by nanomaterial on pests remains unclear. In the present study, both CYP15C1 and C-factor genes were cloned from the midgut of C. suppressalis, and the transcript of C-factor is most highly expressed in heads. Feeding a dsCYP15C1 or dsC-factor - nanomaterial mixture can downregulate the gene expression and significantly increase larval mortality. More importantly, feeding the stacked-dsRNA wrapped by nanomaterial can significantly increase the mortality of C. suppressalis, compared with feeding dsCYP15C1 or dsC-factor - nanomaterial mixture alone. These results showed that CYP15C1 and C-factor could be potential targets for an effective management of C. suppressalis, and we developed a nanoparticle-facilitated stacked-dsRNA strategy in the control of C. suppresallis. Our research provides a theoretical basis for gene function analysis and field pest control, and will promote the application of RNAi technology in the stacked style of pest control.

Schlagwörter:

Animals; Larva/genetics; Moths/genetics; Nanoparticles; RNA Interference; RNA, Double-Stranded/genetics

Kategorien:

1.6 not applicable; 2.2.9 Chilo suppressalis (Rice stem borer); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2.4.1 nanoparticle-mediated dsRNA; 6.1.7 cell differentiation; 6.2.4 molting; 6.2.5.1 metamorphosis; 7.1 Concept development

Zeitschriftenaufsatz

Noriega, Daniel D.; Arraes, Fabricio B. M.; Antonino, José Dijair; Macedo, Leonardo L. P.; Fonseca, Fernando C. A.; Togawa, Roberto C. et al. (2020):

Transcriptome Analysis and Knockdown of the Juvenile Hormone Esterase Gene Reveal Abnormal Feeding Behavior in the Sugarcane Giant Borer.

In: *Frontiers in physiology* 11, S. 588450. DOI: 10.3389/fphys.2020.588450.

Abstract:

The sugarcane giant borer (SGB), Telchin licus licus, is a pest that has strong economic relevance for sugarcane producers. Due to the endophytic behavior of the larva, current methods of management are inefficient. A promising biotechnological management option has been proposed based on RNA interference (RNAi), a process that uses molecules of double-stranded RNA (dsRNA) to specifically knock down essential genes and reduce insect survival. The selection of suitable target genes is often supported by omic sciences. Studies have shown that genes related to feeding adaptation processes are good candidates to be targeted by RNAi for pest management. Among those genes, esterases are highlighted because of their impact on insect development. In this study, the objective was to evaluate the transcriptome responses of the SGB's gut in order to provide curated data of genes that could be used for pest management by RNAi in future studies. Further, we validated the function of an esterase-coding gene and its potential as a target for RNAi-based control. We sequenced the gut transcriptome of SGB larvae by Illumina HiSeq and evaluated its gene expression profiles in response to different diets (sugarcane stalk and artificial diet). We obtained differentially expressed genes (DEGs) involved in detoxification, digestion, and transport, which suggest a generalist mechanism of adaptation in SGB larvae. Among the DEGs, was identified and characterized a candidate juvenile hormone esterase gene (Tljhe). We knocked down the Tljhe gene by oral delivery of dsRNA molecules and evaluated gene expression in the gut. The survival and nutritional parameters of the larvae were measured along the developmental cycle of treated insects. We found that the gene Tljhe acts as a regulator of feeding behavior. The knockdown of Tljhe triggered a forced starvation state in late larval instars that significantly reduced the fitness of the larvae. However, the mechanism of action of this gene remains unclear, and the correlation between the expression of Tljhe and the levels of juvenile hormone (JH) metabolites in the hemolymph of the SGB must be assessed in future research.

Schlagwörter:

insect gut; Lepidoptera/genetics; RNAi; rna-seq; Telchin licus licus

Kategorien:

1.1.6 sugarcane; 2.2.37 Telchin licus licus (Sugarcane giant borer); 3.3 not applicable;
4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.3 not applicable;
6.3.7 feeding behaviour; 7.1 Concept development

Zeitschriftenaufsatz

Nowara, Daniela; Gay, Alexandra; Lacomme, Christophe; Shaw, Jane; Ridout, Christopher; Douchkov, Dimitar et al. (2010):

HIGS: host-induced gene silencing in the obligate biotrophic fungal pathogen Blumeria graminis.

In: *The Plant cell* 22 (9), S. 3130–3141. DOI: 10.1105/tpc.110.077040.

Abstract:

Powdery mildew fungi are obligate biotrophic pathogens that only grow on living hosts and cause damage in thousands of plant species. Despite their agronomical importance, little direct functional evidence for genes of pathogenicity and virulence is currently available because mutagenesis and transformation protocols are lacking. Here, we show that the accumulation in barley (Hordeum vulgare) and wheat (Triticum aestivum) of double-stranded or antisense RNA targeting fungal transcripts affects the development of the powdery mildew fungus Blumeria graminis. Proof of concept for host-induced gene silencing was obtained by silencing the effector gene Avra10, which resulted in reduced fungal development in the absence, but not in the presence, of the matching resistance gene Mla10. The fungus could be rescued from the silencing of Avra10 by the transient expression of a synthetic gene that was resistant to RNA interference (RNAi) due to silent point mutations. The results suggest traffic of RNA molecules from host plants into B. graminis and may lead to an RNAi-based crop protection strategy against fungal pathogens.

Schlagwörter:

Ascomycota/genetics; Gene Expression Regulation, Fungal; Hordeum/genetics/microbiology; Host-Pathogen Interactions; molecular sequence data; Plants, Genetically Modified/genetics/microbiology; RNA Interference; RNA, Antisense/metabolism; RNA, Double-Stranded/metabolism; RNA, Fungal/genetics; RNA, Plant/metabolism; Triticum/genetics/microbiology

Kategorien:

1.1.3 Triticum aestivum (wheat); 1.1.5 Hordeum vulgare (barley); 2.1.14 Blumeria graminis; 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.1 endogenous; 5.1.1 stable transgene; 6.1.3 cell wall elongation; 6.7.4 haustorium formation; 6.7.8 infection mechanisms (virulence factors); 7.1 Concept development

Zeitschriftenaufsatz

Oliveira Filho, Josemar Gonalves de; Da Silva, Guilherme Cruz; Cipriano, Lavnia; Gomes, Mariana; Egea, Mariana Buranelo (2021):

Control of postharvest fungal diseases in fruits using external application of RNAi.

In: *Journal of food science* 86 (8), S. 3341–3348. DOI: 10.1111/1750-3841.15816.

Abstract:

Contamination with a variety of filamentous fungi can cause deterioration of food and agricultural products. Fungal contaminations reduce the quality and the shelf life of fresh fruits and are one of the main causes of economic loss in the global fresh fruit industry. Although chemical fungicides are effective and traditionally used to control postharvest fungal diseases, they are harmful to human health. In this context, use of RNA interference (RNAi)-based fungicides is a promising alternative strategy. Spray-induced gene silencing (SIGS) is an innovative RNAi-based approach for silencing target genes in phytopathogens. This review aims to discuss the recent findings on the use of RNAi-based fungicides to control the postharvest spoilage of fresh fruits. PRACTICAL APPLICATION: Control of postharvest fungal diseases is one of the most important strategies to make food available to consumers longer. In this sense, the external application of RNAi seems to be technologically advantageous and efficient as it helps to maintain the characteristics of plant products. In this sense, this review discussed what is possible to find in the literature regarding this new technology.

Schlagwörter:

Agriculture/methods; Fruit/microbiology; Fungi/drug effects/genetics; Plant Diseases/prevention & control; RNA Interference; RNA/pharmacology

Kategorien:

1.3 Fruits; 2.1 fungi; 3.3 not applicable; 4.3 not applicable; 5.2 exogenous; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Ouyang, Shou-Qiang; Ji, Hui-Min; Feng, Tao; Luo, Shu-Jie; Cheng, Lu; Wang, Nan (2023):

Artificial trans-kingdom RNAi of FolRDR1 is a potential strategy to control tomato wilt disease.

In: *PLoS pathogens* 19 (6), e1011463. DOI: 10.1371/journal.ppat.1011463.

Abstract:

Tomato is cultivated worldwide as a nutrient-rich vegetable crop. Tomato wilt disease caused by Fusarium oxysporum f.sp. Lycopersici (Fol) is one of the most serious fungal diseases posing threats to tomato production. Recently, the development of Spray-Induced Gene Silencing (SIGS) directs a

novel plant disease management by generating an efficient and environmental friendly biocontrol agent. Here, we characterized that FolRDR1 (RNA-dependent RNA polymerase 1) mediated the pathogen invasion to the host plant tomato, and played as an essential regulator in pathogen development and pathogenicity. Our fluorescence tracing data further presented that effective uptakes of FolRDR1-dsRNAs were observed in both Fol and tomato tissues. Subsequently, exogenous application of FolRDR1-dsRNAs on pre-Fol-infected tomato leaves resulted in significant alleviation of tomato wilt disease symptoms. Particularly, FolRDR1-RNAi was highly specific without sequence off-target in related plants. Our results of pathogen gene-targeting RNAi have provided a new strategy for tomato wilt disease management by developing an environmentally-friendly biocontrol agent.

Schlagwörter:

Fusarium/genetics; gene silencing; Plant Diseases/genetics/prevention & control/microbiology; RNA Interference; Solanum lycopersicum/genetics

Kategorien:

1.4.8 Solanum lycopersium (tomato); 2.1.10 Fusarium oxysporum; 3.3 not applicable; 4.3 not applicable; 5.2.2 spray (SIGS); 6.7.8 infection mechanisms (virulence factors); 7.2 Concept demonstration

Zeitschriftenaufsatz

Padilla-Roji, Isabel; Ruiz-Jiménez, Laura; Bakhat, Nisrine; Vielba-Fernández, Alejandra; Pérez-García, Alejandro; Fernández-Ortuño, Dolores (2023):

RNAi Technology: A New Path for the Research and Management of Obligate Biotrophic Phytopathogenic Fungi.

In: *International journal of molecular sciences* 24 (10). DOI: 10.3390/ijms24109082.

Abstract:

Powdery mildew and rust fungi are major agricultural problems affecting many economically important crops and causing significant yield losses. These fungi are obligate biotrophic parasites that are completely dependent on their hosts for growth and reproduction. Biotrophy in these fungi is determined by the presence of haustoria, specialized fungal cells that are responsible for nutrient uptake and molecular dialogue with the host, a fact that undoubtedly complicates their study under laboratory conditions, especially in terms of genetic manipulation. RNA interference (RNAi) is the biological process of suppressing the expression of a target gene through double-stranded RNA that induces mRNA degradation. RNAi technology has revolutionized the study of these obligate biotrophic fungi by enabling the analysis of gene function in these fungal. More importantly, RNAi technology has opened new perspectives for the management of powdery mildew and rust diseases, first through the stable expression of RNAi constructs in transgenic plants and, more recently, through the non-transgenic approach called spray-induced gene silencing (SIGS). In this review, the impact of RNAi technology on the research and management of powdery mildew and rust fungi will be addressed.

Schlagwörter:

Basidiomycota/genetics; Erysiphe; gene silencing; Plant Diseases/genetics/microbiology; RNA Interference; RNA, Double-Stranded/genetics

Kategorien:

1.6 not applicable; 2.1 fungi; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Parsons, Keith H.; Mondal, Mosharrof H.; McCormick, Charles L.; Flynt, Alex S. (2018):

Guanidinium-Functionalized Interpolyelectrolyte Complexes Enabling RNAi in Resistant Insect Pests.

In: *Biomacromolecules* 19 (4), S. 1111–1117. DOI: 10.1021/acs.biomac.7b01717.

Abstract:

RNAi-based technologies are ideal for pest control as they can provide species specificity and spare nontarget organisms. However, in some pests biological barriers prevent use of RNAi, and therefore broad application. In this study we tested the ability of a synthetic cationic polymer, poly-[N-(3-guanidinopropyl)methacrylamide] (pGPMA), that mimics arginine-rich cell penetrating peptides to trigger RNAi in an insensitive animal- Spodoptera frugiperda. Polymer-dsRNA interpolyelectrolyte complexes (IPECs) were found to be efficiently taken up by cells, and to drive highly efficient gene knockdown. These IPECs could also trigger target gene knockdown and moderate larval mortality when fed to S. frugiperda larvae. This effect was sequence specific, which is consistent with the low toxicity we found to be associated with this polymer. A method for oral delivery of dsRNA is critical to development of RNAi-based insecticides. Thus, this technology has the potential to make RNAi-based pest control useful for targeting numerous species and facilitate use of RNAi in pest management practices.

Schlagwörter:

Acrylamides/chemistry/pharmacology; Animals; Guanidine/chemical synthesis/pharmacology; Insecticides/chemistry/pharmacology; Pest Control, Biological; Polyelectrolytes/pharmacology; Polymers/chemistry/pharmacology; RNA Interference/drug effects; Species Specificity; Spodoptera/drug effects/genetics/pathogenicity

Kategorien:

1.6 not applicable; 2.2.13 Spodoptera frugiperda; 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2.1 mRNA degradation; 5.2.4.8 polymer-mediated dsRNA; 6.1.15 intracellular transport; 6.1.17 cell proliferation, mitosis; 6.3 energy metabolism; 7.1 Concept development

Zeitschriftenaufsatz

Paudel, Jamuna Risal; Davidson, Charlotte; Song, Jun; Maxim, Itkin; Aharoni, Asaph; Tai, Helen H. (2017):

Pathogen and Pest Responses Are Altered Due to RNAi-Mediated Knockdown of GLYCOALKALOID METABOLISM 4 in Solanum tuberosum.

In: *Molecular plant-microbe interactions : MPMI* 30 (11), S. 876–885. DOI: 10.1094/MPMI-02-17-0033-R.

Abstract:

Steroidal glycoalkaloids (SGAs) are major secondary metabolites constitutively produced in cultivated potato Solanum tuberosum, and α-solanine and α-chaconine are the most abundant SGAs. SGAs are toxic to humans at high levels but their role in plant protection against pests and pathogens is yet to be established. In this study, levels of SGAs in potato were reduced by RNA interference (RNAi)-mediated silencing of GLYCOALKALOID METABOLISM 4 (GAME4)-a gene encoding cytochrome P450, involved in an oxidation step in the conversion of cholesterol to SGA aglycones. Two GAME4 RNAi lines, T8 and T9, were used to investigate the effects of manipulation of the SGA biosynthetic pathway in potato. Growth and development of an insect pest, Colorado potato beetle (CPB), were affected in these lines. While no effect on CPB leaf consumption or weight gain was observed, early instar larval death and accelerated development of the insect was found while feeding on leaves of GAME4 RNAi lines. Modulation of SGA biosynthetic pathway in GAME4 RNAi plants was associated with a larger alteration to the metabolite profile, including increased levels of one or both the steroidal saponins or phytoecdysteroids, which could affect insect mortality as well as development time. Colonization by Verticillium dahliae on GAME4 RNAi plants was also tested. There were increased pathogen levels in the T8 GAME4 RNAi line but not in the T9. Metabolite differences between T8 and T9 were found and may have contributed to differences in V. dahliae infection. Drought responses created by osmotic stress were not affected by modulation of SGA biosynthetic pathway in potato.

Schlagwörter:

Alkaloids/chemistry/metabolism; Animals; Coleoptera/growth & development/physiology; Droughts; Ecdysteroids/metabolism; Gene Expression Regulation, Plant; Gene Knockdown Techniques; Glycosylation; Metabolome/genetics; metabolomics; Plant Proteins/genetics/metabolism; Plants, Genetically Modified; RNA Interference; RNA, Messenger/genetics/metabolism; Solanum tuberosum/genetics/microbiology/parasitology; Stress, Physiological/genetics; Verticillium/physiology

Kategorien:

1.4.5 Solanum tuberosum (potato); 2.2.19 Leptinotarsa decemlineata (Colorado potato beetle); 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.1 Concept development

Zeitschriftenaufsatz

Prakash M., Navale; Maligeppagol, Manamohan; R., Asokan; V., Krishna; G., Sharath Chandra; K., Prasad Babu et al. (2017):

Transgenic tomato expressing dsRNA of juvenile hormone acid O-methyl transferase gene of Helicoverpa armigera (Lepidoptera: Noctuidae) affects larval growth and its development.

In: *Journal of Asia-Pacific Entomology* 20 (2), S. 559–567.

Abstract:

Helicoverpa annigera is an important pest infesting a number of crops of high commercial value leading to huge economic losses globally. RNA interference (RNAi) is a potent tool for the control of insect pests and towards this objective, transgenic tomato expressing dsRNA of Juvenile hormone acid methyl transferase (JHAMT) gene of H. annigera was developed. The H. annigera larvae fed on HaJHAMT tomato leaves led to a severe (90%) down regulation of the cognate gene expression thus adversely affected the feeding and metamorphosis. Reduction in larval and pupal weight and inability to undergo complete metamorphosis were observed that led to larval-pupal intermediates and subsequently affected the eclosion. Thus, JH down regulation is an attractive target due to specificity to insects and its important role in insect growth and development to engineer insect resistance in crops using RNAi. (C) 2017 Korean Society of Applied Entomology, Taiwan Entomological Society and Malaysian Plant Protection Society. Published by Elsevier B.V.

Schlagwörter:

cotton bollworm (Helicoverpa armigera); gene silencing; insect resistance; juvenile hormone acid methyl transferase; metamorphosis; RNAi (RNA interference); tomato (Solanum lycopersicum)

Kategorien:

1.4.8 Solanum lycopersium (tomato); 2.2.1 Helicoverpa armigera (Cotton bollworm/Chickpea pod borer); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2.1 mRNA degradation; 5.1 endogenous; 5.1.1 stable transgene; 6.2.4 molting; 6.2.5.1 metamorphosis; 7.2 Concept demonstration

Zeitschriftenaufsatz

Prentice, Katterinne; Smagghe, Guy; Gheysen, Godelieve; Christiaens, Olivier (2019):

Nuclease activity decreases the RNAi response in the sweetpotato weevil Cylas puncticollis.

In: *Insect biochemistry and molecular biology* 110, S. 80–89.

Abstract:

RNA interference (RNAi) refers to the process of suppression of gene expression in eukaryotes, which has a great potential for the control of pest and diseases. Unfortunately, the efficacy of this technology is limited or at best variable in some insects. In the African sweet potato weevil (SPW) Cylas puncticollis, a devastating pest that affects the sweet potato production in Sub-Saharan Africa

(SSA), the RNAi response was highly efficient when dsRNA was delivered by injection, but it showed a reduced response by oral feeding. In the present study, the role of nucleases in the reduced RNAi efficiency in C. puncticollis is investigated. Several putative dsRNases were first identified in the transcriptome of the SPW through homology search and were subsequently further characterized. Two of these dsRNases were specifically expressed in the gut tissue of the insect and we could demonstrate through RNAi experiments that these affected dsRNA stability in the gut. Furthermore, RNAi-of-RNAi studies, using snf7 as a reporter gene, demonstrated that silencing one of these nucleases, Cp-dsRNase-3, clearly increases RNAi efficacy. After silencing this nuclease, significantly higher mortality was observed in dssnf7-treated insects 14 days post-feeding as compared to control treatments, and the gene downregulation was confirmed at the transcript level via qPCR analysis. Taken together, our results demonstrate that the RNAi efficiency is certainly impaired by nuclease activity in the gut environment of the SPW Cylas puncticollis.

Schlagwörter:

Amino Acid Sequence; Animals; Endonucleases/chemistry/genetics/metabolism; Insect Proteins/chemistry/genetics/metabolism; Phylogeny; RNA Interference; RNA, Double-Stranded/pharmacology; Sequence Alignment; Transcriptome; Weevils/enzymology/genetics/metabolism

Kategorien:

1.6 not applicable; 2.2.33 Cylas puncticollis (Sweetpotato weevil); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2 exogenous; 6.5.2 RNAi machinery; 7.1 Concept development

Zeitschriftenaufsatz

Qiao, Lulu; Lan, Chi; Capriotti, Luca; Ah-Fong, Audrey; Nino Sanchez, Jonatan; Hamby, Rachael et al. (2021):

Spray-induced gene silencing for disease control is dependent on the efficiency of pathogen RNA uptake.

In: *Plant Biotechnol J* 19 (9), S. 1756–1768. DOI: 10.1111/pbi.13589.

Abstract:

Recent discoveries show that fungi can take up environmental RNA, which can then silence fungal genes through environmental RNA interference. This discovery prompted the development of Spray-Induced Gene Silencing (SIGS) for plant disease management. In this study, we aimed to determine the efficacy of SIGS across a variety of eukaryotic microbes. We first examined the efficiency of RNA uptake in multiple pathogenic and non-pathogenic fungi, and an oomycete pathogen. We observed efficient double-stranded RNA (dsRNA) uptake in the fungal plant pathogens Botrytis cinerea, Sclerotinia sclerotiorum, Rhizoctonia solani, Aspergillus niger and Verticillium dahliae, but no uptake in Colletotrichum gloeosporioides, and weak uptake in a beneficial fungus, Trichoderma virens. For the oomycete plant pathogen, Phytophthora infestans, RNA uptake was limited and varied across different cell types and developmental stages. Topical application of dsRNA targeting virulence-

related genes in pathogens with high RNA uptake efficiency significantly inhibited plant disease symptoms, whereas the application of dsRNA in pathogens with low RNA uptake efficiency did not suppress infection. Our results have revealed that dsRNA uptake efficiencies vary across eukaryotic microbe species and cell types. The success of SIGS for plant disease management can largely be determined by the pathogen's RNA uptake efficiency.

Schlagwörter:

Ascomycota; Botrytis; Colletotrichum; gene silencing; Plant Diseases; Rhizoctonia; RNA Interference; RNA, Double-Stranded/genetics

Kategorien:

1.6 not applicable; 2.1 fungi; 3.3 not applicable; 4.1.2 intermolecular dsRNA; 5.2.2 spray (SIGS); 6.9 not applicable; 7.1 Concept development

Zeitschriftenaufsatz

Qiao, Lulu; Niño-Sánchez, Jonatan; Hamby, Rachael; Capriotti, Luca; Chen, Angela; Mezzetti, Bruno; Jin, Hailing (2023):

Artificial nanovesicles for dsRNA delivery in spray-induced gene silencing for crop protection.

In: *Plant Biotechnol J*. DOI: 10.1111/pbi.14001.

Abstract:

Spray-induced gene silencing (SIGS) is an innovative and eco-friendly technology where topical application of pathogen gene-targeting RNAs to plant material can enable disease control. SIGS applications remain limited because of the instability of RNA, which can be rapidly degraded when exposed to various environmental conditions. Inspired by the natural mechanism of cross-kingdom RNAi through extracellular vesicle trafficking, we describe herein the use of artificial nanovesicles (AVs) for RNA encapsulation and control against the fungal pathogen, Botrytis cinerea. AVs were synthesized using three different cationic lipid formulations, DOTAP + PEG, DOTAP and DODMA, and examined for their ability to protect and deliver double stranded RNA (dsRNA). All three formulations enabled dsRNA delivery and uptake by B. cinerea. Further, encapsulating dsRNA in AVs provided strong protection from nuclease degradation and from removal by leaf washing. This improved stability led to prolonged RNAi-mediated protection against B. cinerea both on pre- and post-harvest plant material using AVs. Specifically, the AVs extended the protection duration conferred by dsRNA to 10 days on tomato and grape fruits and to 21 days on grape leaves. The results of this work demonstrate how AVs can be used as a new nanocarrier to overcome RNA instability in SIGS for crop protection.

Schlagwörter:

dsRNA; liposomes; Nanoparticles; RNAi (RNA interference); SIGS (spray-induced gene silencing); small RNA; uptake efficiency

Kategorien:

1.3.2 Vitis vinifera (grape); 1.4.4 Lactuca sativa (Garden lettuce); 1.4.8 Solanum lycopersium (tomato); 1.5.1 "ornamental plants"; 2.1.2 Botrytis cinerea; 3.3 not applicable; 4.1.2 intermolecular

dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2.2 spray (SIGS); 5.2.4.4 artificial nanovesicle encapsulation; 6.7.8 infection mechanisms (virulence factors); 7.2 Concept demonstration

Zeitschriftenaufsatz

Ramon, Matthew; Devos, Yann; Lanzoni, Anna; Liu, Yi; Gomes, Ana; Gennaro, Andrea; Waigmann, Elisabeth (2014):

RNAi-based GM plants: food for thought for risk assessors.

In: *Plant Biotechnol J* 12 (9), S. 1271–1273. DOI: 10.1111/pbi.12305.

Abstract:

RNA interference (RNAi) is an emerging technology that offers new opportunities for the generation of new traits in genetically modified (GM) plants. Potential risks associated with RNAi-based GM plants and issues specific to their risk assessment were discussed during an international scientific workshop (June 2014) organized by the European Food Safety Authority (EFSA). Selected key outcomes of the workshop are reported here.

Schlagwörter:

food safety; Plants, Genetically Modified/genetics; Risk Assessment; RNA Interference; RNA, Double-Stranded/metabolism

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Rank, Aline Pereira; Koch, Aline (2021):

Lab-to-Field Transition of RNA Spray Applications - How Far Are We?

In: *Frontiers in Plant Science* 12, S. 755203. DOI: 10.3389/fpls.2021.755203.

Abstract:

The drastic loss of biodiversity has alarmed the public and raised sociopolitical demand for chemical pesticide-free plant production, which is now treated by governments worldwide as a top priority. Given this global challenge, RNAi-based technologies are rapidly evolving as a promising substitute to conventional chemical pesticides. Primarily, genetically modified (GM) crops expressing double-stranded (ds)RNA-mediating gene silencing of foreign transcripts have been developed. However, since the cultivation of GM RNAi crops is viewed negatively in numerous countries, GM-free exogenous RNA spray applications attract tremendous scientific and political interest. The sudden rise in demand for pesticide alternatives has boosted research on sprayable RNA biopesticides, generating significant technological developments and advancing the potential for field applications in the near future. Here we review the latest advances that could pave the way for a quick lab-to-

field transition for RNA sprays, which, as safe, selective, broadly applicable, and cost-effective biopesticides, represent an innovation in sustainable crop production. Given these latest advances, we further discuss technological limitations, knowledge gaps in the research, safety concerns and regulatory requirements that need to be considered and addressed before RNA sprays can become a reliable and realistic agricultural approach.

Schlagwörter:

dsRNA; nanomaterial-based siRNA delivery; RNA biopesticides; RNAi-based plant protection; SIGS (spray-induced gene silencing); siRNA

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.2.2 spray (SIGS); 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Ražná, Katarína; Cagáň, Ľudovít (2019):

The Role of MicroRNAs in Genome Response to Plant-Lepidoptera Interaction.

In: *Plants (Basel, Switzerland)* 8 (12). DOI: 10.3390/plants8120529.

Abstract:

RNA interference is a known phenomenon of plant immune responses, involving the regulation of gene expression. The key components triggering the silencing of targeted sequences are double-stranded RNA molecules. The regulation of host-pathogen interactions is controlled by miRNA molecules, which regulate the expression of host resistance genes or the genes of the pathogen. The review focused on basic principles of RNA interference as a gene-silencing-based defense mechanism and the role of miRNA molecules in insect genomes. RNA interference as a tool for plant protection management is discussed. The review summarizes current miRNA-based biotechnology approaches for plant protection management.

Schlagwörter:

host-lepidoptera interaction; microRNA; RNAi

Kategorien:

1.6 not applicable; 2.2 insects; 3.3 not applicable; 4.1.1.1 miRNA; 4.2.1 transcriptional gene regulation; 4.2.2 post-transcriptional gene regulation; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Rebijith, K. B.; Asokan, R.; Hande, H. Ranjitha; Kumar, N. K. Krishna; Krishna, V.; Vinutha, J.; Bakthavatsalam, N. (2016):

RNA Interference of Odorant-Binding Protein 2 (OBP2) of the Cotton Aphid, Aphis gossypii (Glover), Resulted in Altered Electrophysiological Responses.

In: *Applied biochemistry and biotechnology* 178 (2), S. 251–266. DOI: 10.1007/s12010-015-1869-7.

Abstract:

Aphis gossypii (Glover) (Hemiptera: Aphididae) is a highly invasive pest that feeds primarily on phloem resulting in severe economic loss to growers. A. gossypii has cosmopolitan distribution with broad host range, polyphenism, parthenogenetic mode of reproduction, vectoring abilities, and host alteration which has profound influence on its management. Odorant-binding proteins (OBPs) in insects are involved in olfaction, playing a key role in orienting the insect for feeding or oviposition. Recent studies revealed that OBP2 is found in both sensilla trichodea and sensilla basiconica and is preferentially binds to plant volatiles, thus playing crucial roles in host-seeking, detection of oviposition attractants, etc., However, information about the role of OBP2 in A. gossypii (AgOBP2) is still unavailable. In this study, we cloned and characterized OBP2, ortholog from A. gossypii, and the full-length AgOBP2 complementary DNA (cDNA) consisted of 859 bp with an open reading frame of 732 bp. Phylogenetic analysis resulted in grouping of AgOBP2 protein with members of the tribe Aphidini. Further, diet-mediated delivery of double-stranded RNA for AgOBP2 induced silencing, which was evaluated at 48 and 96 h. The reverse transcriptase real-time quantitative polymerase chain reaction (RTq-PCR) results revealed that the level of AgOBP2 messenger RNA (mRNA) was significantly reduced (55-77 %) in dsAgOBP2 treatment after 96 h as compared to the untreated control. The same was reiterated by the electrophysiological responses in the aphids which was reduced (>50 % at 0.25 μg/μl concentration) as compared to the untreated control. Thus, our results showed the potential of gene silencing, possibly to interfere with the odorant perception of A. gossypii for RNAi-mediated pest management. The results from our study provided the first evidence that AgOBP2 play crucial roles in host-seeking, detection of oviposition attractants, etc.; as a result, we suggests that OBP2 could potentially serve as a practicable target for RNAi-mediated gene silencing in hemipteran insect pest control.

Schlagwörter:

Amino Acid Sequence; Animals; Aphids/classification/genetics/physiology; gene silencing; Insect Proteins/chemistry/genetics; molecular sequence data; Pest Control, Biological; Phylogeny; Receptors, Odorant/chemistry/genetics; RNA Interference; Sequence Homology, Amino Acid

Kategorien:

1.6 not applicable; 2.2.18 Aphis gossypii; 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2.1 mRNA degradation; 5.2 exogenous; 6.6.2 olfaction; 7.1 Concept development

Zeitschriftenaufsatz

Romeis, Jörg; Widmer, Franco (2020):

Assessing the Risks of Topically Applied dsRNA-Based Products to Non-target Arthropods.

In: *Frontiers in Plant Science* 11, S. 679. DOI: 10.3389/fpls.2020.00679.

Abstract:

RNA interference (RNAi) is a powerful technology that offers new opportunities for pest control through silencing of genes that are essential for the survival of arthropod pests. The approach relies on sequence-specificity of applied double-stranded (ds) RNA that can be designed to have a very narrow spectrum of both the target gene product (RNA) as well as the target organism, and thus allowing highly targeted pest control. Successful RNAi has been reported from a number of arthropod species belonging to various orders. Pest control may be achieved by applying dsRNA as foliar sprays. One of the main concerns related to the use of dsRNA is adverse environmental effects particularly on valued non-target species. Arthropods form an important part of the biodiversity in agricultural landscapes and contribute important ecosystem services. Consequently, environmental risk assessment (ERA) for potential impacts that plant protection products may have on valued non-target arthropods is legally required prior to their placement on the market. We describe how problem formulation can be used to set the context and to develop plausible pathways on how the application of dsRNA-based products could harm valued non-target arthropod species, such as those contributing to biological pest control. The current knowledge regarding the exposure to and the hazard posed by dsRNA in spray products for non-target arthropods is reviewed and suggestions are provided on how to select the most suitable test species and to conduct laboratory-based toxicity studies that provide robust, reliable and interpretable results to support the ERA.

Schlagwörter:

risk assessment strategies

Kategorien:

1.6 not applicable; 2.7 not applicable; 4.3 not applicable; 5.2 exogenous; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Rosa, Cristina; Kuo, Yen-Wen; Wuriyanghan, Hada; Falk, Bryce W. (2018):

RNA Interference Mechanisms and Applications in Plant Pathology.

In: *Annual review of phytopathology* 56, S. 581–610. DOI: 10.1146/annurev-phyto-080417-050044.

Abstract:

The origin of RNA interference (RNAi), the cell sentinel system widely shared among eukaryotes that recognizes RNAs and specifically degrades or prevents their translation in cells, is suggested to predate the last eukaryote common ancestor (138). Of particular relevance to plant pathology is that in plants, but also in some fungi, insects, and lower eukaryotes, RNAi is a primary and effective antiviral defense, and recent studies have revealed that small RNAs (sRNAs) involved in RNAi play important roles in other plant diseases, including those caused by cellular plant pathogens. Because of this, and because RNAi can be manipulated to interfere with the expression of endogenous genes in an intra- or interspecific manner, RNAi has been used as a tool in studies of gene function but also for plant protection. Here, we review the discovery of RNAi, canonical mechanisms, experimental and translational applications, and new RNA-based technologies of importance to plant pathology.

Schlagwörter:

Plant Diseases/genetics/microbiology; Plant Pathology; RNA Interference

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Roy, Amit; George, Smitha; Palli, Subba Reddy (2017):

Multiple functions of CREB-binding protein during postembryonic development: identification of target genes.

In: *BMC genomics* 18 (1), S. 996. DOI: 10.1186/s12864-017-4373-3.

Abstract:

BACKGROUND

Juvenile hormones (JH) and ecdysteroids control postembryonic development in insects. They serve as valuable targets for pest management. Hence, understanding the molecular mechanisms of their action is of crucial importance. CREB-binding protein (CBP) is a universal transcriptional co-regulator. It controls the expression of several genes including those from hormone signaling pathways through co-activation of many transcription factors. However, the role of CBP during postembryonic development in insects is not well understood. Therefore, we have studied the role of CBP in postembryonic development in Tribolium, a model coleopteran insect.

RESULTS

CBP is ubiquitously expressed in the red flour beetle, Tribolium castaneum. RNA interference (RNAi) mediated knockdown of CBP resulted in a decrease in JH induction of Kr-h1 gene expression in Tribolium larvae and led to a block in their development. Moreover, the injection of CBP double-stranded RNA (dsRNA) showed lethal phenotypes within 8 days of injection. RNA-seq and subsequent differential gene expression analysis identified CBP target genes in Tribolium. Knockdown of CBP

caused a decrease in the expression of 1306 genes coding for transcription factors and other proteins associated with growth and development. Depletion of CBP impaired the expression of several JH response genes (e.g., Kr-h1, Hairy, early trypsin) and ecdysone response genes (EcR, E74, E75, and broad complex). Further, GO enrichment analyses of the downregulated genes showed enrichment in different functions including developmental processes, pigmentation, anatomical structure development, regulation of biological and cellular processes, etc.

CONCLUSION

These data suggest diverse but crucial roles for CBP during postembryonic development in the coleopteran model insect, Tribolium. It can serve as a target for RNAi mediated pest management of this stored product pest.

Schlagwörter:

Animals; CREB-Binding Protein/antagonists & inhibitors/genetics/metabolism/physiology; gene expression; Insect Proteins/antagonists & inhibitors/genetics/metabolism/physiology; Juvenile Hormones/pharmacology; Larva/genetics/metabolism; RNA Interference; Tribolium/genetics/growth & development/metabolism

Kategorien:

1.6 not applicable; 2.2.12 Tribolium castaneum (Red flour beetle); 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.2.4 molting; 6.2.5.1 metamorphosis; 6.4.2 endocrine system; 7.1 Concept development

Zeitschriftenaufsatz

Ruiz-Jiménez, Laura; Polonio, Álvaro; Vielba-Fernández, Alejandra; Pérez-García, Alejandro; Fernández-Ortuño, Dolores (2021):

Gene Mining for Conserved, Non-Annotated Proteins of Podosphaera xanthii Identifies Novel Target Candidates for Controlling Powdery Mildews by Spray-Induced Gene Silencing.

In: *Journal of fungi (Basel, Switzerland)* 7 (9). DOI: 10.3390/jof7090735.

Abstract:

The powdery mildew fungus Podosphaera xanthii is one of the most important limiting factors for cucurbit production worldwide. Despite the significant efforts made by breeding and chemical companies, effective control of this pathogen remains elusive to growers. In this work, we examined the suitability of RNAi technology called spray-induced gene silencing (SIGS) for controlling cucurbit powdery mildew. Using leaf disc and cotyledon infiltration assays, we tested the efficacy of dsRNA applications to induce gene silencing in P. xanthii. Furthermore, to identify new target candidate genes, we analyzed sixty conserved and non-annotated proteins (CNAPs) deduced from the P. xanthii transcriptome in silico. Six proteins presumably involved in essential functions, specifically respiration (CNAP8878, CNAP9066, CNAP10905 and CNAP30520), glycosylation (CNAP1048) and efflux transport (CNAP948), were identified. Functional analysis of these CNAP coding genes by dsRNA-induced gene

silencing resulted in strong silencing phenotypes with large reductions in fungal growth and disease symptoms. Due to their important contributions to fungal development, the CNAP1048, CNAP10905 and CNAP30520 genes were selected as targets to conduct SIGS assays under plant growth chamber conditions. The spray application of these dsRNAs induced high levels of disease control, supporting that SIGS could be a sustainable approach to combat powdery mildew diseases.

Schlagwörter:

cucurbits; dsRNA; Podosphaera xanthii; powdery mildews; RNAi (RNA interference); SIGS (spray-induced gene silencing)

Kategorien:

1.4.1 Cucurbita pepo (zucchini); 1.4.2 Cucumis melo (melon); 2.1.13 Podosphaera xanthii; 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2.2 spray (SIGS); 6.1.15 intracellular transport; 6.3.3 glycosylation; 6.3.4 respiration; 7.2 Concept demonstration

Zeitschriftenaufsatz

Rutter, Brian D.; Innes, Roger W. (2018):

Extracellular vesicles as key mediators of plant-microbe interactions.

In: *Current Opinion in Plant Biology* 44, S. 16–22. DOI: 10.1016/j.pbi.2018.01.008.

Abstract:

Extracellular vesicles (EVs) are lipid compartments capable of trafficking proteins, lipids, RNA and metabolites between cells. Plant cells have been shown to secrete EVs during immune responses, but virtually nothing is known about their formation, contents or ultimate function. Recently developed methods for isolating plant EVs have revealed that these EVs are enriched in stress response proteins and signaling lipids, and appear to display antifungal activity. Comparison to work on animal EVs, and the observation that host-derived small interfering RNAs and microRNAs can silence fungal genes, suggests that plant EVs may also mediate trans-kingdom RNA interference. Many fundamental questions remain, however, regarding how plant EVs are produced, how they move, and if and how they are taken up by target cells.

Schlagwörter:

Extracellular Vesicles/genetics/metabolism; MicroRNAs/genetics/metabolism; RNA Interference; RNA, Small Interfering/genetics/metabolism

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.2.4.2 extracellular vesicle mediated; 6.9 not applicable; 7.1 Concept development

Zeitschriftenaufsatz

S, Sundaresha; Sharma, Sanjeev; Bairwa, Aarti; Tomar, Maharishi; Kumar, Ravinder; Bhardwaj, Vinay et al. (2022):

Spraying of dsRNA molecules derived from Phytophthora infestans, along with nanoclay carriers as a proof of concept for developing novel protection strategy for potato late blight.

In: *Pest management science* 78 (7), S. 3183–3192. DOI: 10.1002/ps.6949.

Abstract:

BACKGROUND

Phytophthora infestans is a late blight-causing oomycetes pathogen. It rapidly evolves and adapts to the host background and new fungicide molecules within a few years of their release, most likely because of the predominance of transposable elements in its genome. Frequent applications of fungicides cause environmental concerns. Here, we developed target-specific RNA interference (RNAi)-based molecules, along with nanoclay carriers, that when sprayed on plants are capable of effectively reducing late blight infection.

RESULTS

Targeted the genes unique to sporulation, early satge infection and the metabolism pathway stages based on in an our own microarray data. We used nanoclay as a carrier for sorbitol dehydrogenase, heat shock protein 90, translation elongation factor 1-α, phospholipase-D like 3 and glycosylphosphatidylinositol-anchored acidic serine-threonine-rich HAM34-like protein double-stranded (ds)RNAs, which were assessed by culture bioassay, detached leaf assay and spray methods, and revealed a reduction in growth, sporulation and symptom expression. Plants sprayed with multigene targeted dsRNA-nanoclay showed enhanced disease resistance (4% disease severity) and less sporulation (<1 × 103) compared with plants sprayed with dsRNA alone.

CONCLUSION

The use of nanoclay with multigene targeted dsRNA was assumed to be involved in effective delivery, protection and boosting the action of RNAi as a spray-induced gene silencing approach (SIGS). A significant reduction in growth, sporulation, disease severity and decreased gene expression authenticates the effects of SIGS on late blight progression. This study demonstrated as a proof of concept the dsRNA-nanoclay SIGS approach, which could be used as an alternative to chemical fungicides and transgenic approaches to develop an environmentally friendly novel plant protection strategy for late blight. © 2022 Society of Chemical Industry.

Schlagwörter:

Disease Resistance/genetics; Fungicides, Industrial/pharmacology; Phytophthora infestans/genetics; Plant Diseases/prevention & control; RNA, Double-Stranded/genetics; Solanum tuberosum/genetics

Kategorien:

1.4.5 Solanum tuberosum (potato); 2.1.6 Phytophthora infestans; 3.3 not applicable;
4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2.2 spray (SIGS);

5.2.4.6 nanoclay-carrier dsRNA; 6.1.5 translation; 6.3.8 metabolism; 6.7.8 infection mechanisms (virulence factors); 7.2 Concept demonstration

Zeitschriftenaufsatz

Saakre, Manjesh; Jaiswal, Sandeep; Rathinam, Maniraj; Raman, K. Venkat; Tilgam, Jyotsana; Paul, Krishnayan et al. (2023):

Host-Delivered RNA Interference for Durable Pest Resistance in Plants: Advanced Methods, Challenges, and Applications.

In: *Molecular biotechnology*. DOI: 10.1007/s12033-023-00833-9.

Abstract:

Insect-pests infestation greatly affects global agricultural production and is projected to become more severe in upcoming years. There is concern about pesticide application being ineffective due to insect resistance and environmental toxicity. Reduced effectiveness of Bt toxins also made the scientific community shift toward alternative strategies to control devastating agricultural pests. With the advent of host-delivered RNA interference, also known as host-induced gene silencing, targeted insect genes have been suppressed through genetic engineering tools to deliver a novel insect-pest resistance strategy for combating a number of agricultural pests. This review recapitulates the possible mechanism of host-delivered RNA interference (HD-RNAi), in particular, the silencing of target genes of insect-pests. We emphasize the development of the latest strategies against evolving insect targets including designing of artificial microRNAs, vector constructs, and the benefit of using plastid transformation to transform target RNA-interfering genes. Advantages of using HD-RNAi over other small RNA delivery modes and also the supremacy of HD-RNAi over the CRISPR-Cas system particularly for insect resistance have been described. However, the broader application of this technology is restricted due to its several limitations. Using artificial miRNA designs, the host-delivered RNAi + Bt combinatorial approach and chloroplast transformation can overcome limitations of RNAi. With careful design and delivery approaches, RNAi promises to be extremely valuable and effective plant protection strategy to attain durable insect-pest resistance in crops. Development of transgenic plant using novel strategies to achieve durable resistance against the target insect.

Schlagwörter:

artificial micro RNAs; chloroplast transformation; durable resistance; HIGS (host-induced gene silencing); RNAi+Bt

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.1 endogenous; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Salvador, Ricardo; Maskin, Laura; Niz, José; Turica, Mariana; Pedarros, Analía; Hopp, Esteban; Lewi, Dalia (2022):

RNAi Expression in Cotton for Control of Herbivorous Insects.

In: *Methods in molecular biology (Clifton, N.J.)* 2360, S. 217–233. DOI: 10.1007/978-1-0716-1633-8_17.

Abstract:

Cultivated cotton (Gossypium hirsutum) is heavily attacked by various species of insects worldwide and breeding of new varieties resistant to pests is still a hard battle to win. RNAi technology is an important reverse genetics tool to induce gene silencing in eukaryotic organisms and produce phenotypic modifications. In cotton, RNAi was applied to investigate gene function and enhance resistance to insects and pathogens. Different methods and techniques can be used to synthetize double stranded RNA (dsRNA) into plant cells. The Agrobacterium-mediated transformation is a common method to introduce RNAi binary plasmids into cotton genome and obtain stable transgenics plants. This methodology includes the coculture of cotton tissues with Agrobacterium cultures, selection of transgenic cells and induction of somatic embryogenesis to finally obtain transgenic plants after a relatively long period of time. The transient synthesis of dsRNA mediated by virus-induced gene silencing (VIGS) in cotton is an alternative to anticipate the silencing effect of a specific RNA sequence, prior to the development of a stable transgenic plant. VIGS vectors are incorporated into the plant by agroinfiltration technique. During VIGS replication inside plant cells, synthetized dsRNA allows the study on specific heterologous gene expression including the phenotypic effect on herbivorous target pests, thus facilitating a rapid evaluation of dsRNA expressed in cotton plants against individual insect target genes. Here we describe the complementation of these two techniques to evaluate RNAi-based cotton plant protection against insect pests.

Schlagwörter:

Agrobacterium/genetics; Animals; control pests; Gossypium/genetics; Insecta; plant breeding; Plants, Genetically Modified/genetics; RNA Interference; RNA, Double-Stranded/genetics; RNAi (RNA interference); transgenic cotton plants; virus-induced gene silencing (VIGS)

Kategorien:

1.2.2 Gossypium hirsutum (cotton); 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.1.1 stable transgene; 5.2.1 VIGS; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Samarskaya, Viktoriya O.; Spechenkova, Nadezhda; Markin, Nikolay; Suprunova, Tatyana P.; Zavriev, Sergey K.; Love, Andrew J. et al. (2022):

Impact of Exogenous Application of Potato Virus Y-Specific dsRNA on RNA Interference, Pattern-Triggered Immunity and Poly(ADP-ribose) Metabolism.

In: *International journal of molecular sciences* 23 (14). DOI: 10.3390/ijms23147915.

Abstract:

In this work we developed and exploited a spray-induced gene silencing (SIGS)-based approach to deliver double-stranded RNA (dsRNA), which was found to protect potato against potato virus Y (PVY) infection. Given that dsRNA can act as a defence-inducing signal that can trigger sequence-specific RNA interference (RNAi) and non-specific pattern-triggered immunity (PTI), we suspected that these two pathways may be invoked via exogeneous application of dsRNA, which may account for the alterations in PVY susceptibility in dsRNA-treated potato plants. Therefore, we tested the impact of exogenously applied PVY-derived dsRNA on both these layers of defence (RNAi and PTI) and explored its effect on accumulation of a homologous virus (PVY) and an unrelated virus (potato virus X, PVX). Here, we show that application of PVY dsRNA in potato plants induced accumulation of both small interfering RNAs (siRNAs), a hallmark of RNAi, and some PTI-related gene transcripts such as WRKY29 (WRKY transcription factor 29; molecular marker of PTI), RbohD (respiratory burst oxidase homolog D), EDS5 (enhanced disease susceptibility 5), SERK3 (somatic embryogenesis receptor kinase 3) encoding brassinosteroid-insensitive 1-associated receptor kinase 1 (BAK1), and PR-1b (pathogenesis-related gene 1b). With respect to virus infections, PVY dsRNA suppressed only PVY replication but did not exhibit any effect on PVX infection in spite of the induction of PTI-like effects in the presence of PVX. Given that RNAi-mediated antiviral immunity acts as the major virus resistance mechanism in plants, it can be suggested that dsRNA-based PTI alone may not be strong enough to suppress virus infection. In addition to RNAi- and PTI-inducing activities, we also showed that PVY-specific dsRNA is able to upregulate production of a key enzyme involved in poly(ADP-ribose) metabolism, namely poly(ADP-ribose) glycohydrolase (PARG), which is regarded as a positive regulator of biotic stress responses. These findings offer insights for future development of innovative approaches which could integrate dsRNA-induced RNAi, PTI and modulation of poly(ADP-ribose) metabolism in a co-ordinated manner, to ensure a high level of crop protection.

Schlagwörter:

Plant Diseases/genetics; Poly Adenosine Diphosphate Ribose; Potyvirus/physiology; RNA Interference; RNA, Double-Stranded/genetics; RNA, Small Interfering/genetics/metabolism; Solanum tuberosum/metabolism

Kategorien:

1.4.5 Solanum tuberosum (potato); 2.4.5 Potato virus Y (PVY); 3.3 not applicable;
4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2.2 spray (SIGS); 6.9 not applicable; 7.1 Concept development

Zeitschriftenaufsatz

Sarkar, Atrayee; Roy-Barman, Subhankar (2021):

Spray-Induced Silencing of Pathogenicity Gene MoDES1 via Exogenous Double-Stranded RNA Can Confer Partial Resistance Against Fungal Blast in Rice.

In: *Frontiers in Plant Science* 12, S. 733129. DOI: 10.3389/fpls.2021.733129.

Abstract:

Over the past years, RNA interference (RNAi) has been used as a promising combat strategy against a wide range of pests and pathogens in ensuring global food security. It involves the induction of highly specific posttranscriptional regulation of target essential genes from an organism, via the application of precursor long, non-coding double-stranded RNA (dsRNA) molecules that share sequence-complementarity with the mRNAs of the targets. Fungal blast disease caused by Magnaporthe oryzae is one of the most deadly diseases of rice and wheat incurring huge losses in global crop yield. To date, the host-induced gene silencing (HIGS) and virus-induced gene silencing (VIGS) aspects of RNAi have been successfully exploited in developing resistance against M. oryzae in rice. Spray-induced gene silencing (SIGS) is a current, potential, non-transformative, and environment-friendly pest and pathogen management strategy, where naked or nanomaterial-bound dsRNA are sprayed on leaves to cause selective knockdown of pathogenicity genes. Although it relies on the ability of fungal pathogens to uptake sprayed RNA, its efficiency varies largely across phytopathogens and their genes, targeted for silencing. Here, we report a transient dsRNA supplementation system for the targeted knockdown of MoDES1, a host-defense suppressor pathogenicity gene from M. oryzae. We validate the feasibility of in vivo SIGS and post-uptake transfer of RNA signals to distal plant parts in rice-M. oryzae pathosystem through a GFP-based reporter system. A protocol for efficient silencing via direct foliar spray of naked dsRNA was optimized. As proof-of-concept, we demonstrate the phenotypic impacts of in vitro dsDES1 treatment on growth, conidiation, ROS-scavenging ability, and pathogenic potential of M. oryzae. Furthermore, our extrapolatory dsDES1 spray experiments on wounded leaves and whole rice plants indicate resultant silencing of MoDES1 that conferred significant resistance against the fungal blast disease. The evaluation of primary and secondary host defense responses provides evidence supporting the notion that spray of sequence-specific dsRNA on wounded leaf tissue can cause systemic and sustained silencing of a M. oryzae target gene. For the first time, we establish a transgene-free SIGS approach as a promising crop protection strategy against the notorious rice-blast fungus.

Schlagwörter:

disease resistance; dsRNA; fungal blast; MoDES1; rice (Oryza sativa); RNAi-based plant protection; SIGS (spray-induced gene silencing)

Kategorien:

1.1.4 Oryza sativa (rice); 2.1.8 Magnaporthe oryzae; 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.1.2 transient transgene; 5.2.2 spray (SIGS); 6.7.8 infection mechanisms (virulence factors); 7.2 Concept demonstration

Zeitschriftenaufsatz

Schlemmer, Timo; Barth, Patrick; Weipert, Lisa; Preußer, Christian; Hardt, Martin; Möbus, Anna et al. (2021):

Isolation and Characterization of Barley (Hordeum vulgare) Extracellular Vesicles to Assess Their Role in RNA Spray-Based Crop Protection.

In: *International journal of molecular sciences* 22 (13). DOI: 10.3390/ijms22137212.

Abstract:

The demonstration that spray-induced gene silencing (SIGS) can confer strong disease resistance, bypassing the laborious and time-consuming transgenic expression of double-stranded (ds)RNA to induce the gene silencing of pathogenic targets, was ground-breaking. However, future field applications will require fundamental mechanistic knowledge of dsRNA uptake, processing, and transfer. There is increasing evidence that extracellular vesicles (EVs) mediate the transfer of transgene-derived small interfering (si)RNAs in host-induced gene silencing (HIGS) applications. In this study, we establish a protocol for barley EV isolation and assess the possibilities for EVs regarding the translocation of sprayed dsRNA from barley (Hordeum vulgare) to its interacting fungal pathogens. We found barley EVs that were 156 nm in size, containing predominantly 21 and 19 nucleotide (nts) siRNAs, starting with a 5'-terminal Adenine. Although a direct comparison of the RNA cargo between HIGS and SIGS EV isolates is improper given their underlying mechanistic differences, we identified sequence-identical siRNAs in both systems. Overall, the number of siRNAs isolated from the EVs of dsRNA-sprayed barley plants with sequence complementarity to the sprayed dsRNA precursor was low. However, whether these few siRNAs are sufficient to induce the SIGS of pathogenic target genes requires further research. Taken together, our results raise the possibility that EVs may not be mandatory for the spray-delivered siRNA uptake and induction of SIGS.

Schlagwörter:

Crop Protection/methods; Cytochrome P450 Family 3/genetics; Disease Resistance/genetics; Extracellular Vesicles/genetics/microbiology; gene silencing; Hordeum/genetics/microbiology; Host Microbial Interactions/genetics; Plant Diseases/genetics/microbiology/prevention & control; RNA Interference; RNA, Plant/genetics/isolation & purification; RNA, Small Interfering/administration & dosage/isolation & purification

Kategorien:

1.1.5 Hordeum vulgare (barley); 2.1 fungi; 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2.2 spray (SIGS); 5.2.4.2 extracellular vesicle mediated; 6.9 not applicable; 7.1 Concept development

Zeitschriftenaufsatz

Schlemmer, Timo; Lischka, Richard; Wegner, Linus; Ehlers, Katrin; Biedenkopf, Dagmar; Koch, Aline (2022):

Extracellular vesicles isolated from dsRNA-sprayed barley plants exhibit no growth inhibition or gene silencing in Fusarium graminearum.

In: *Fungal biology and biotechnology* 9 (1), S. 14. DOI: 10.1186/s40694-022-00143-w.

Abstract:

Numerous reports have shown that incorporating a double-stranded RNA (dsRNA)-expressing transgene into plants or applying dsRNA by spraying it onto their leaves successfully protects them against invading pathogens exploiting the mechanism of RNA interference (RNAi). How dsRNAs or siRNAs are transferred between donor host cells and recipient fungal cells is largely unknown. It is speculated that plant extracellular vesicles (EVs) function as RNA shuttles between plants and their pathogens. Recently, we found that EVs isolated from host-induced gene silencing (HIGS) or spray-induced gene silencing (SIGS) plants contained dsRNA-derived siRNAs. In this study, we evaluated whether isolated EVs from dsRNA-sprayed barley (Hordeum vulgare) plants affected the growth of the phytopathogenic ascomycete Fusarium graminearum. Encouraged by our previous finding that dropping barley-derived EVs on F. graminearum cultures caused fungal stress phenotypes, we conducted an in vitro growth experiment in microtiter plates where we co-cultivated F. graminearum with plant EVs isolated from dsRNA-sprayed barley leaves. We observed that co-cultivation of F. graminearum macroconidia with barley EVs did not affect fungal growth. Furthermore, plant EVs containing SIGS-derived siRNA appeared not to affect F. graminearum growth and showed no gene silencing activity on F. graminearum CYP51 genes. Based on our findings, we concluded that either the amount of SIGS-derived siRNA was insufficient to induce target gene silencing in F. graminearum, indicating that the role of EVs in SIGS is minor, or that F. graminearum uptake of plant EVs from liquid cultures was inefficient or impossible.

Schlagwörter:

barley; dsRNA; dsRNA-based pesticides; extracellular vesicles; Fusarium graminearium; plant EVs; RNAi (RNA interference); RNAi-based plant protection; SIGS (spray-induced gene silencing); siRNA

Kategorien:

1.1.5 Hordeum vulgare (barley); 2.1.1 Fusarium graminearum; 3.3 not applicable;
4.1.2 intermolecular dsRNA; 5.2.2 spray (SIGS); 5.2.4.2 extracellular vesicle mediated; 6.9 not applicable; 7.1 Concept development

Zeitschriftenaufsatz

Šečić, Ena; Kogel, Karl-Heinz; Ladera-Carmona, Maria Jose (2021):

Biotic stress-associated microRNA families in plants.

In: *Journal of Plant Physiology* 263, S. 153451. DOI: 10.1016/j.jplph.2021.153451.

Abstract:

Plants and animals utilize various regulatory mechanisms for control of gene expression during development in different tissues and cell types. About 30 years ago, a new mechanism of gene regulation, termed RNA interference (RNAi), was discovered and proved revolutionary for the mechanistic understanding of gene regulation. Noncoding RNAs, including short, 21-24 nucleotide (nt) long microRNAs (miRNAs), endogenously-generated from MIR genes, are key components of RNAi processes, by post-transcriptionally controlling transcripts with antisense complementarity through either translational repression or mRNA degradation. Since their discovery, important roles in regulation of ontogenetic development, cell differentiation, proliferation, and apoptosis in eukaryotes have been elucidated. In plants, miRNAs are known regulatory elements of basic endogenous functions and responses to the environmental stimuli. While the role of miRNAs in regulation of nutrient uptake, circadian clock and general response to abiotic stress is already well understood, a comprehensive understanding of their immune-regulatory roles in response to various biotic stress factors has not yet been achieved. This review summarizes the current understanding of the function of miRNAs and their targets in plants during interaction with microbial pathogens and symbionts. Additionally, we provide a consensus conclusion regarding the typical induction or repression response of conserved miRNA families to pathogenic and beneficial fungi, bacteria, and oomycetes, as well as an outlook of agronomic application of miRNAs in plants. Further investigation of plant miRNAs responsive to microbes, aided with novel sequencing and bioinformatics approaches for discovery and prediction in non-model organisms holds great potential for development of new forms of plant protection.

Schlagwörter:

Gene Expression Regulation, Plant; MicroRNAs/genetics; Plant Development/genetics; Plant Growth Regulators/genetics; RNA, Plant; Stress, Physiological/genetics/physiology

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Šečić, Ena; Zanini, Silvia; Kogel, Karl-Heinz (2019):

Further Elucidation of the Argonaute and Dicer Protein Families in the Model Grass Species Brachypodium distachyon.

In: *Frontiers in Plant Science* 10, S. 1332. DOI: 10.3389/fpls.2019.01332.

Abstract:

RNA interference (RNAi) is a biological process in which small RNAs regulate gene silencing at the transcriptional or posttranscriptional level. The trigger for gene silencing is double-stranded RNA generated from an endogenous genomic locus or a foreign source, such as a transgene or virus. In addition to regulating endogenous gene expression, RNAi provides the mechanistic basis for small RNA-mediated communication between plant hosts and interacting pathogenic microbes, known as cross-kingdom RNAi. Two core protein components, Argonaute (AGO) and Dicer (DCL), are central to the RNAi machinery of eukaryotes. Plants encode for several copies of AGO and DCL genes; in Arabidopsis thaliana, the AGO protein family contains 10 members, and the DCL family contains four. Little is known about the conservation and specific roles of these proteins in monocotyledonous plants, which account for the most important food staples. Here, we utilized in silico tools to investigate the structure and related functions of AGO and DCL proteins from the model grass Brachypodium distachyon. Based on the presence of characteristic domains, 16 BdAGO- and 6 BdDCL-predicted proteins were identified. Phylogenetic analysis showed that both protein families were expanded in Brachypodium as compared with Arabidopsis. For BdDCL proteins, both plant species contain a single copy of DCL1 and DCL4; however, Brachypodium contains two copies each of DCL2 and DCL3. Members of the BdAGO family were placed in all three functional clades of AGO proteins previously described in Arabidopsis. The greatest expansion occurred in the AtAGO1/5/10 clade, which contains nine BdAGOs (BdAGO5/6/7/9/10/11/12/15/16). The catalytic tetrad of the AGO P-element-induced wimpy testis domain (PIWI), which is required for endonuclease activity, is conserved in most BdAGOs, with the exception of BdAGO1, which lacks the last D/H residue. Three-dimensional modeling of BdAGO proteins using tertiary structure prediction software supported the phylogenetic classification. We also predicted a provisional interactome network for BdAGOs, their localization within the cell, and organ/tissue-specific expression. Exploring the specifics of RNAi machinery proteins in a model grass species can serve as a proxy for agronomically important cereals such as barley and wheat, where the development of RNAi-based plant protection strategies is of great interest.

Schlagwörter:

argonaute; Brachypodium; dicer; prediction; protein; RNAi (RNA interference); structure

Kategorien:

1.5.2 Brachypodium distachyon; 1.5.3 Arabidopsis thaliana; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.3.3 glycosylation; 7.3 not applicable

Zeitschriftenaufsatz

Sharma, Rohit; Taning, Clauvis Nji Tizi; Smagghe, Guy; Christiaens, Olivier (2021):

Silencing of Double-Stranded Ribonuclease Improves Oral RNAi Efficacy in Southern Green Stinkbug Nezaraviridula.

In: *Insects* 12 (2). DOI: 10.3390/insects12020115.

Abstract:

Variability in RNA-interference (RNAi) efficacy among different insect orders poses a big hurdle in the development of RNAi-based pest control strategies. The activity of double-stranded ribonucleases (dsRNases) in the digestive canal of insects can be one of the critical factors affecting oral RNAi efficacy. Here, the involvement of these dsRNases in the southern green stinkbug Nezaraviridula was investigated. First, the full sequence of the only dsRNase (NvdsRNase) in the transcriptome of N. viridula was obtained, followed by an oral feeding bioassay to evaluate the effect of NvdsRNase-silencing on oral RNAi efficacy. The NvdsRNase was first silenced in nymphs by NvdsRNase-dsRNA injections, followed by exposure to an artificial diet containing a lethal αCop-specific dsRNA. A significantly higher mortality was observed in the NvdsRNase-silenced nymphs when placed on the dsαCop-containing diet (65%) than in the dsGFP injected and dsαCop fed control (46.67%). Additionally, an ex vivo dsRNA degradation assay showed a higher stability of dsRNA in the saliva and midgut juice of NvdsRNase-silenced adults. These results provide evidence for the involvement of NvdsRNase in the reduction of oral RNAi efficacy in N. viridula. This information will be useful in further improving potential RNAi-based strategies to control this pest.

Schlagwörter:

dsRNA; N. viridula; NvdsRNase; oral feeding bioassay; RNAi of RNAi; saliva and midgut juice

Kategorien:

1.6 not applicable; 2.2.3 Nezara viridula (Southern green stinkbug); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2 exogenous; 6.5.2 RNAi machinery; 7.1 Concept development

Zeitschriftenaufsatz

Shelby, Emily A.; Moss, Jeanette B.; Andreason, Sharon A.; Simmons, Alvin M.; Moore, Allen J.; Moore, Patricia J. (2020):

Debugging: Strategies and Considerations for Efficient RNAi-Mediated Control of the Whitefly Bemisia tabaci.

In: *Insects* 11 (11). DOI: 10.3390/insects11110723.

Abstract:

The whitefly Bemisia tabaci is a globally important pest that is difficult to control through insecticides, transgenic crops, and natural enemies. Post-transcriptional gene silencing through RNA interference (RNAi) has shown potential as a pest management strategy against B. tabaci. While genomic data and other resources are available to create highly effective customizable pest management strategies with RNAi, current applications do not capitalize on species-specific biology. This lack of specificity has the potential to have substantial ecological impacts. Here, we discuss both short- and long-term considerations for sustainable RNAi pest management strategies for B. tabaci, focusing on the need for species specificity incorporating both life history and population genetic considerations. We provide a conceptual framework for selecting sublethal target genes based on their involvement in physiological pathways, which has the greatest potential to ameliorate unintended negative consequences. We suggest that these considerations allow an integrated pest management approach, with fewer negative ecological impacts and reduced likelihood of the evolution of resistant populations.

Schlagwörter:

Bemisia tabaci; integrated pest management; RNAi; RNAi (RNA interference); whiteflies

Kategorien:

1.6 not applicable; 2.2.10 Bemisia tabaci (White fly); 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Shim, Jae-Kyoung; Lee, Gwan-Seok; Lee, Sukchan; Lee, Kyeong-Yeoll (2015):

Oral ingestion of heat shock protein 70 dsRNA is lethal under normal and thermal stress conditions in the sweetpotato whitefly, Bemisia tabaci.

In: *Journal of Asia-Pacific Entomology* 18 (4), S. 797–800. DOI: 10.1016/j.aspen.2015.10.004.

Abstract:

The sweetpotato whitefly, Bemisia tabaci, is a serious pest of various horticultural crops worldwide. Since 1990s, B. tabaci has been invaded into many countries due to climate change and global trades

of agricultural products. Due to the rapid development of pesticide resistance, alternative techniques have been required for the control of B. tabaci. Here, we investigated whether oral ingestion of dsRNA induced knockdown in whiteflies. Double stranded RNA (dsRNA) targeting heat shock protein 70 (hsp70) mRNA was produced from cDNA using specific primers. Adult whiteflies (less than 12 h post-eclosion) were allowed to ingest 20% (w/v) sucrose solution containing hsp70 dsRNA in a 2-layered membrane feeding tube. Individual whiteflies ingested 16.9 ng dsRNA on an average in 24 h. Quantitative real-time PCR analysis showed that dsRNA ingestion decreased the mRNA level of hsp70 in a dose-dependent manner, and the hsp70 knockdown was sustained for at least 3 days. Furthermore, dsRNA-treated whiteflies showed increased mortality after 3 days of incubation at 25 degrees C. Mortality was prompted by heat shock but not by cold shock treatments. Our data suggest that the oral delivery of dsRNA had excellent efficacy for the RNAi treatment of whiteflies and that the expression of hsp70 was critical for the survival of B. tabaci, regardless of the temperature conditions.

Schlagwörter:

environmental stresses; gene regulation; heat shock proteins; RNAi (RNA interference); whiteflies

Kategorien:

1.6 not applicable; 2.2.10 Bemisia tabaci (White fly); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2.1 mRNA degradation; 5.2 exogenous; 6.1.5 translation; 7.1 Concept development

Zeitschriftenaufsatz

Song, Xiu-Shi; Gu, Kai-Xin; Duan, Xiao-Xin; Xiao, Xue-Mei; Hou, Yi-Ping; Duan, Ya-Bing et al. (2018):

Secondary amplification of siRNA machinery limits the application of spray-induced gene silencing.

In: *Molecular plant pathology* 19 (12), S. 2543–2560. DOI: 10.1111/mpp.12728.

Abstract:

Spray-induced gene silencing (SIGS) is an innovative strategy for crop protection. However, the mechanism of SIGS is not known. Here, we first demonstrate that secondary small interfering RNA (siRNA) amplification limits the application of SIGS. A myosin5 gene (Myo5) was chosen as the target of SIGS in an agronomically important pathogen-Fusarium asiaticum. Five segments corresponding to the different regions of the Myo5 gene were found to efficiently silence Myo5, resulting in cell wall defects, life cycle disruption and virulence reduction. Myo5-8 (one of the Myo5 segments) induced sequence-specific RNA interference (RNAi) activity in F. asiaticum, F. graminearum, F. tricinctum and F. oxysporum, but not in other fungi, in vitro. Remarkably, the silencing of Myo5 lasted for only 9 h unless the double-stranded RNA (dsRNA) was continuously supplied, because F. asiaticum is unable to maintain siRNA amplification. After spraying on plants, dsRNAs were more efficiently taken up via the wounded surface. The antifungal activity of dsRNAs taken up by plant cells was higher and longer lasting than that dried onto the plant surface. In contrast with dsRNAs in fungi, dsRNAs in plant cells could efficiently turn into substantial siRNAs via secondary amplification machinery. Our findings

provide new implications to develop SIGS as a mainstream disease control strategy against Fusarium and other fungi.

Schlagwörter:

Arabidopsis/microbiology; Cell Wall/metabolism; Chitin/metabolism; Disease Resistance/genetics; Fusarium/genetics/metabolism/pathogenicity; Gene Expression Regulation, Fungal; Gene Knockdown Techniques; gene silencing; Hyphae/metabolism/ultrastructure; Myosins/genetics; Plant Cells/microbiology; Plant Diseases/microbiology; Reproduction; RNA, Double-Stranded/metabolism; RNA, Small Interfering/metabolism; Spores, Fungal/metabolism/ultrastructure; Transformation, Genetic; Triticum/microbiology; Virulence

Kategorien:

1.6 not applicable; 2.1.7 Fusarium spp.; 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.1.2.4 secondary RNAi; 4.2.2 post-transcriptional gene regulation; 5.2.2 spray (SIGS); 6.1.15 intracellular transport; 7.1 Concept development

Zeitschriftenaufsatz

Spada, Maria; Pugliesi, Claudio; Fambrini, Marco; Palpacelli, Diego; Pecchia, Susanna (2023):

Knockdown of Bmp1 and Pls1 Virulence Genes by Exogenous Application of RNAi-Inducing dsRNA in Botrytis cinerea.

In: *International journal of molecular sciences* 24 (5). DOI: 10.3390/ijms24054869.

Abstract:

Botrytis cinerea is a pathogen of wide agronomic and scientific importance partly due to its tendency to develop fungicide resistance. Recently, there has been great interest in the use of RNA interference as a control strategy against B. cinerea. In order to reduce the possible effects on non-target species, the sequence-dependent nature of RNAi can be used as an advantage to customize the design of dsRNA molecules. We selected two genes related to virulence: BcBmp1 (a MAP kinase essential for fungal pathogenesis) and BcPls1 (a tetraspanin related to appressorium penetration). After performing a prediction analysis of small interfering RNAs, dsRNAs of 344 (BcBmp1) and 413 (BcPls1) nucleotides were synthesized in vitro. We tested the effect of topical applications of dsRNAs, both in vitro by a fungal growth assay in microtiter plates and in vivo on artificially inoculated detached lettuce leaves. In both cases, topical applications of dsRNA led to gene knockdown with a delay in conidial germination for BcBmp1, an evident growth retardation for BcPls1, and a strong reduction in necrotic lesions on lettuce leaves for both genes. Furthermore, a strongly reduced expression of the BcBmp1 and BcPls1 genes was observed in both in vitro and in vivo experiments, suggesting that these genes could be promising targets for the development of RNAi-based fungicides against B. cinerea.

Schlagwörter:

Botrytis; Fungicides, Industrial/pharmacology; Plant Diseases/microbiology; RNA Interference; RNA, Double-Stranded/metabolism; Virulence/genetics

Kategorien:

1.4.4 Lactuca sativa (Garden lettuce); 2.1.2 Botrytis cinerea; 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2.1 mRNA degradation; 5.2 exogenous; 6.7.6 appressorium formation; 6.7.8 infection mechanisms (virulence factors); 7.2 Concept demonstration

Zeitschriftenaufsatz

Spada, Maria; Pugliesi, Claudio; Fambrini, Marco; Pecchia, Susanna (2021):

Silencing of the Slt2-Type MAP Kinase Bmp3 in Botrytiscinerea by Application of Exogenous dsRNA Affects Fungal Growth and Virulence on Lactuca sativa.

In: *International journal of molecular sciences* 22 (10). DOI: 10.3390/ijms22105362.

Abstract:

Botrytis cinerea can attack over 500 genera of vascular plants and is considered the second phytopathogen in the 'top ten' for its economic importance. Traditional fungicides can be ineffective and with increasing fungicide resistance, new sustainable technologies are required. Lately, RNA interference-based fungicides are emerging for their potential uses in crop protection. Therefore, we assessed the potential of this innovative approach targeting the MAP kinase Bmp3 in B. cinerea, a gene involved in saprophytic growth, response to low osmolarity, conidiation, surface sensing, host penetration and lesion formation. After performing a prediction analysis of small interfering RNAs, a 427 nucleotides long dsRNA was selected as construct. We tested the effect of topical applications of dsRNA construct both in vitro by a fungal growth assay in microtiter plates and in vivo on detached lettuce leaves artificially inoculated. In both cases, topical applications of dsRNA led to gene knockdown with a delay in conidial germination, an evident growth retardation and a strong reduction of necrotic lesions on leaves. These results correlated with a strongly reduced expression of Bmp3 gene. In accordance to these findings, the Bmp3 gene could be a promising target for the development of an RNAi-based fungicide against B. cinerea.

Schlagwörter:

Bone Morphogenetic Protein 3/genetics/metabolism; Botrytis/genetics/metabolism/pathogenicity; Fungicides, Industrial/metabolism; Lettuce/genetics/microbiology; Plant Diseases/microbiology; Plant Leaves/microbiology; RNA, Double-Stranded/metabolism/pharmacology; RNA, Small Interfering/genetics/pharmacology; Virulence

Kategorien:

1.4.4 Lactuca sativa (Garden lettuce); 2.1.2 Botrytis cinerea; 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2 exogenous; 6.5.1 lesion formation; 6.7.2 host penetration; 6.7.3 surface sensing; 6.8.1 conidiation; 7.2 Concept demonstration

Zeitschriftenaufsatz

Sundararajan, Poorva; Kalyandurg, Pruthvi B.; Liu, Qinsong; Chawade, Aakash; Whisson, Stephen C.; Vetukuri, Ramesh R. (2022):

Spray-Induced Gene Silencing to Study Gene Function in Phytophthora.

In: *Methods in molecular biology (Clifton, N.J.)* 2536, S. 459–474. DOI: 10.1007/978-1-0716-2517-0_27.

Abstract:

RNA interference (RNAi) is a conserved cellular defense mechanism mediated by double-stranded RNA (dsRNA) that can regulate gene expression through targeted destruction of mRNAs (messenger RNAs). Recent studies have shown that spraying dsRNAs or small RNAs (sRNAs) that target essential genes of pathogens on plant surfaces can confer protection against pests and pathogens. Also called spray-induced gene silencing (SIGS), this strategy can be used for disease control and for transient gene silencing to study the function of genes in plant-pathogen interactions. Furthermore, as sRNAs can move locally, systemically, and cross-kingdom during plant-microbe interactions, SIGS allows quick detection and characterization of gene functions in pathogens and plants.

Schlagwörter:

gene silencing; Phytophthora/genetics; Plants/genetics; RNA Interference; RNA, Double-Stranded/genetics

Kategorien:

1.6 not applicable; 2.1 fungi; 3.3 not applicable; 4.3 not applicable; 5.2.2 spray (SIGS); 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Sundaresha, Siddappa; Bairwa, Aarti; Tomar, Maharishi; Kumar, Ravinder; Venkatasalam, E. P.; Sagar, Vinay et al. (2022):

In Vitro Method for Synthesis of Large-Scale dsRNA Molecule as a Novel Plant Protection Strategy.

In: *Methods in molecular biology (Clifton, N.J.)* 2408, S. 211–226. DOI: 10.1007/978-1-0716-1875-2_14.

Abstract:

Double-stranded RNA (dsRNAs) molecules are the precursors and effective triggers of RNAi in most organisms. RNAi can be induced by the direct introduction of dsRNAs in plants, fungi, insects, and nematodes. Until now RNAi is usually established by transformation of the plant with a construct that produces hairpin RNAs. Alternatively, advances in RNA biology demonstrated efficiently the in vitro method of large-scale synthesis of dsRNA molecule. Here we describe the de novo synthesis of dsRNA molecule targeting the specific gene of interest for functional application. Selection of off-target effective siRNA regions, flanking of T7 promoter sequences, T7 polymerase reaction, and

maintenance of the stability of dsRNA molecules are the main criteria of this method to obtain pure and effective yield for functional applications. IPTG (isopropyl-β-D-thiogalactopyranoside) induced, T7 express E. coli cells, could be used for large scale synthesis of dsRNA molecule are also described in this method.

Schlagwörter:

Escherichia coli/genetics; Plants/genetics; RNA Interference; RNA, Double-Stranded/genetics; RNA, Small Interfering

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Taning, Clauvis Nt; Arpaia, Salvatore; Christiaens, Olivier; Dietz-Pfeilstetter, Antje; Jones, Huw; Mezzetti, Bruno et al. (2020):

RNA-based biocontrol compounds: current status and perspectives to reach the market.

In: *Pest management science* 76 (3), S. 841–845. DOI: 10.1002/ps.5686.

Abstract:

Facing current climate challenges and drastically reduced chemical options for plant protection, the exploitation of RNA interference (RNAi) as an agricultural biotechnology tool has unveiled possible new solutions to the global problems of agricultural losses caused by pests and other biotic and abiotic stresses. While the use of RNAi as a tool in agriculture is still limited to a few transgenic crops, and only adopted in restricted parts of the world, scientists and industry are already seeking innovations in leveraging and exploiting the potential of RNAi in the form of RNA-based biocontrol compounds for external applications. Here, we highlight the expanding research and development pipeline, commercial landscape and regulatory environment surrounding the pursuit of RNA-based biocontrol compounds with improved environmental profiles. The commitments of well-established agrochemical companies to invest in research endeavours and the role of start-up companies are crucial for the successful development of practical applications for these compounds. Additionally, the availability of standardized guidelines to tackle regulatory ambiguities surrounding RNA-based biocontrol compounds will help to facilitate the entire commercialization process. Finally, communication to create awareness and public acceptance will be key to the deployment of these compounds. © 2019 Society of Chemical Industry.

Schlagwörter:

agriculture; biocontrol; biosafety; biotechnology; Crops, Agricultural; dsRNA; regulatory; RNA; RNA Interference; RNAi (RNA interference)

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.2 exogenous; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Tenllado, Francisco; Llave, César; Daz-Ruz, José Ramn (2004):

RNA interference as a new biotechnological tool for the control of virus diseases in plants.

In: *Virus research* 102 (1), S. 85–96. DOI: 10.1016/j.virusres.2004.01.019.

Abstract:

RNA silencing occurs in a wide variety of organisms, including protozoa, fungi, plants and animals and involves recognition of a target RNA and initiation of a sequence-specific RNA degradation pathway in the cytoplasm. In the last few years, there have been considerable advances in our understanding of post-transcriptional gene silencing (PTGS). This mechanism is conceived as a natural antiviral defense system in plants that is activated as a response to double-stranded RNA (dsRNA) formed during virus replication. To develop new approaches for plant protection against virus diseases based on PTGS we have expanded previous findings on RNA interference (RNAi) in animals by using dsRNA to specifically interfere with virus infection in plants. This approach differs from strategies based on transgenic expression of RNAs but still relies on PTGS as a means to achieve pathogen-derived resistance (PDR). Our findings suggest that exogenously supplied dsRNA could form the basis for the development of an environmentally safe, new biotechnological tool aimed at protecting crops against virus diseases, provided that some limitations of the current status of the approach could be overcome.

Schlagwörter:

Biotechnology/methods; Crops, Agricultural; MicroRNAs/genetics/metabolism; Plant Diseases/virology; Plant Viruses/genetics/physiology; RNA Interference; RNA Processing, Post-Transcriptional; RNA Stability; RNA, Double-Stranded/metabolism

Kategorien:

1.6 not applicable; 2.4 viruses; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Thakur, Nidhi; Upadhyay, Santosh Kumar; Verma, Praveen C.; Chandrashekar, Krishnappa; Tuli, Rakesh; Singh, Pradhyumna K. (2014):

Enhanced whitefly resistance in transgenic tobacco plants expressing double stranded RNA of v-ATPase A gene.

In: *PloS one* 9 (3), e87235. DOI: 10.1371/journal.pone.0087235.

Abstract:

BACKGROUND

Expression of double strand RNA (dsRNA) designed against important insect genes in transgenic plants have been shown to give protection against pests through RNA interference (RNAi), thus opening the way for a new generation of insect-resistant crops. We have earlier compared the efficacy of dsRNAs/siRNAs, against a number of target genes, for interference in growth of whitefly (Bemisia tabaci) upon oral feeding. The v-ATPase subunit A (v-ATPaseA) coding gene was identified as a crucial target. We now report the effectiveness of transgenic tobacco plants expressing siRNA to silence v-ATPaseA gene expression for the control of whitefly infestation.

METHODOLOGY/PRINCIPAL FINDINGS

Transgenic tobacco lines were developed for the expression of long dsRNA precursor to make siRNA and knock down the v-ATPaseA mRNA in whitefly. Molecular analysis and insecticidal properties of the transgenic plants established the formation of siRNA targeting the whitefly v-ATPaseA, in the leaves. The transcript level of v-ATPaseA in whiteflies was reduced up to 62% after feeding on the transgenic plants. Heavy infestation of whiteflies on the control plants caused significant loss of sugar content which led to the drooping of leaves. The transgenic plants did not show drooping effect.

CONCLUSIONS/SIGNIFICANCE

Host plant derived pest resistance was achieved against whiteflies by genetic transformation of tobacco which generated siRNA against the whitefly v-ATPaseA gene. Transgenic tobacco lines expressing dsRNA of v-ATPaseA, delivered sufficient siRNA to whiteflies feeding on them, mounting a significant silencing response, leading to their mortality. The transcript level of the target gene was reduced in whiteflies feeding on transgenic plants. The strategy can be taken up for genetic engineering of plants to control whiteflies in field crops.

Schlagwörter:

Animals; Base Sequence; Blotting, Northern; DNA Primers; Hemiptera/genetics; Host-Parasite Interactions; Plants, Genetically Modified; Real-Time Polymerase Chain Reaction; RNA, Double-Stranded/genetics; RNA, Small Interfering/genetics; Tobacco/genetics/parasitology; Vacuolar Proton-Translocating ATPases/genetics

Kategorien:

1.2.1 Nicotiana tabacum (tobacco plant); 2.2.10 Bemisia tabaci (White fly); 3.3 not applicable; 4.1.2.2 long non-coding dsRNA; 4.2.2 post-transcriptional gene regulation; 5.1 endogenous; 5.1.1 stable transgene; 6.3 energy metabolism; 7.2 Concept demonstration

Zeitschriftenaufsatz

Tian, Lixia; Zeng, Yang; Xie, Wen; Wu, Qingjun; Wang, Shaoli; Zhou, Xuguo; Zhang, Youjun (2019):

Genome-wide identification and analysis of genes associated with RNA interference in Bemisia tabaci.

In: *Pest management science* 75 (11), S. 3005–3014. DOI: 10.1002/ps.5415.

Abstract:

BACKGROUND

As a method of RNA-mediated gene silencing, RNA interference (RNAi) is a useful reverse genetic tool with which to study gene function, and holds great promise for pest management. Bemisia tabaci is a cosmopolitan pest that causes extensive damage to crops. The mechanism underlying RNAi efficiency in B. tabaci is not well known. We identified and analyzed candidate genes in the RNAi pathway to understand the RNAi mechanism and provide a basis for the application of RNAi in pest management.

RESULTS

We identified 33 genes putatively involved in the RNAi pathway from the B. tabaci Q genome. Phylogenetic and structural analyses confirmed the characteristics of these genes. Furthermore, quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) and transcriptomic analysis profiled gene expression patterns during different developmental stages. Gene expression levels estimated by qRT-PCR and RNA-seq analyses were significantly correlated. Moreover, gene functions were verified by RNAi. When accompanied by knockdown of AGO2, Dicer2 and Sid1, the efficiency of CYP6DB3 RNAi decreased correspondingly.

CONCLUSION

In this study, we annotated and validated genes involved in B. tabaci RNAi. A better understanding of the building blocks of the RNAi process in B. tabaci facilitates integration of this novel biotechnology into the management of this emerging pest, either directly or indirectly. © 2019 Society of Chemical Industry.

Schlagwörter:

Animals; Female; gene expression; Gene Expression Profiling; Genes, Insect; Genome-Wide Association Study; Hemiptera/genetics/growth & development; Male; Nymph/genetics/growth & development; Phylogeny; RNA Interference

Kategorien:

1.6 not applicable; 2.2.10 Bemisia tabaci (White fly); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 5.2 exogenous; 6.9 not applicable; 7.1 Concept development

Zeitschriftenaufsatz

Tian, Tian; Ji, Rui; Fu, Jianmei; Li, Jing; Wang, Lu; Zhang, Hao et al. (2021):

A salivary calcium-binding protein from Laodelphax striatellus acts as an effector that suppresses defense in rice.

In: *Pest management science* 77 (5), S. 2272–2281. DOI: 10.1002/ps.6252.

Abstract:

BACKGROUND

Calcium (Ca2+)-binding proteins in the saliva of herbivorous insects function as effectors to attenuate host plant defenses and thus improve insect feeding performance. Silencing these genes

via transgenic plant-mediated RNAi is thus a promising pest control strategy. However, their sequences and functions in the small brown planthopper Laodelphax striatellus (SBPH) remain to be investigated.

RESULTS

We identified a putative EF-hand Ca2+ -binding protein (LsECP1) in SBPH watery saliva. LsECP1 was expressed extremely high in the salivary glands but at a low level during the egg stage. Transient LsECP1 expression in rice cells indicated its cytoplasm and nucleus localization. The bacterially expressed recombinant LsECP1 protein exhibited Ca2+ -binding activity. Rice plants fed by SBPH nymphs with knocked down LsECP1 exhibited higher levels of cytosolic Ca2+ , jasmonic acid (JA), jasmonoyl-isoleucine (JA-Ile) and hydrogen peroxide (H2 O2). Consistently, application of heterogeneously expressed LsECP1 protein suppressed wound-induced JA, JA-Ile and H2 O2 accumulation in rice. Thus, LsECP1 knockdown by dsRNA injection resulted in reduced feeding, fecundity and survival rates of SBPH reared on rice plants. Transgenic rice plants constitutively expressing LsECP1 dsRNA were produced, and plant-mediated LsECP1 knockdown enhanced rice resistance to SBPH.

CONCLUSION

SBPH LsECP1 acts as an effector to impair host rice defense responses and promotes SBPH performance. This discovery provides a potential gene target for plant-mediated RNAi-based pest management.

Schlagwörter:

Animals; Calcium-Binding Proteins; Hemiptera/genetics; Oryza/genetics; Plants, Genetically Modified/genetics; RNA Interference

Kategorien:

1.1.4 Oryza sativa (rice); 2.2.5 Laodelphax striatellus (Brown planthopper); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.1.1 stable transgene; 6.3.1 feeding efficiency (salivary proteins); 7.2 Concept demonstration

Zeitschriftenaufsatz

Travella, Silvia; Keller, Beat (2009):

Down-regulation of gene expression by RNA-induced gene silencing.

In: *Methods in molecular biology (Clifton, N.J.)* 478, S. 185–199. DOI: 10.1007/978-1-59745-379-0_12.

Abstract:

Down-regulation of endogenous genes via post-transcriptional gene silencing (PTGS) is a key to the characterization of gene function in plants. Many RNA-based silencing mechanisms such as post-transcriptional gene silencing, co-suppression, quelling, and RNA interference (RNAi) have been discovered among species of different kingdoms (plants, fungi, and animals). One of the most interesting discover ies was RNAi, a sequence-specific gene-silencing mechanism initiated by the introduction of double-stranded RNA (dsRNA), homologous in sequence to the silenced gene, which triggers degradation of mRNA. Infection of plants with modified viruses can also induce RNA silencing and is referred to as virus-induced gene silencing (VIGS). In contrast to insertional mutagenesis, these

emerging new reverse genetic approaches represent a powerful tool for exploring gene function and for manipulating gene expression experimentally in cereal species such as barley and wheat. We examined how RNAi and VIGS have been used to assess gene function in barley and wheat, including molecular mechanisms involved in the process and available methodological elements, such as vectors, inoculation procedures, and analysis of silenced phenotypes.

Schlagwörter:

DNA, Complementary/genetics; Down-Regulation; Gene Knockdown Techniques; gene silencing; Genes, Plant/genetics; Genetic Vectors; Hordeum/genetics; Inverted Repeat Sequences; Mosaic Viruses/metabolism; Oxidoreductases/genetics; phenotype; Plasmids/genetics; Poaceae/genetics; Polymerase Chain Reaction; RNA, Messenger/genetics; RNA/genetics/metabolism; Triticum/genetics

Kategorien:

1.1.3 Triticum aestivum (wheat); 1.1.5 Hordeum vulgare (barley); 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.2.1 VIGS; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Tretiakova, Polina; Voegele, Ralf Thomas; Soloviev, Alexander; Link, Tobias Immanuel (2022):

Successful Silencing of the Mycotoxin Synthesis Gene TRI5 in Fusarium culmorum and Observation of Reduced Virulence in VIGS and SIGS Experiments.

In: *Genes* 13 (3). DOI: 10.3390/genes13030395.

Abstract:

Crops constantly experience various biotic stresses during their life cycle, and Fusarium spp. remain one of the most serious groups of pathogens affecting plants. The ability to manipulate the expression of certain microorganism genes via RNAi creates the opportunity for new-generation dsRNA-based preparations to control a large number of diseases. In this study, we applied virus-induced gene silencing (VIGS), and spray-induced gene silencing (SIGS) to silence the trichothecene-producing gene TRI5 in F. culmorum as a means to reduce its aggressiveness on spring wheat. Treatment of the fungus with dsTRI5RNA in vitro reduced deoxynivalenol (DON) and 3-acetyldeoxynivalenol (3-A-DON) accumulations by 53-85% and 61-87%, respectively, and reduced TRI5 expression by 84-97%. VIGS decreased the proportion of infected wheat spikelets by 73%, but upregulation was observed for TRI5. SIGS on wheat leaves and ears using certain dsTRI5RNA amounts negatively impacted F. culmorum growth. However, when performing in vivo analyses of TRI5 mRNA levels, the upregulation of the gene was determined in the variants where fungal colonization was restricted, suggesting a compensatory reaction of the pathogen to RNAi.

Schlagwörter:

Fusarium; Plant Diseases/genetics/microbiology; Triticum; Virulence/genetics

Kategorien:

1.1.3 Triticum aestivum (wheat); 2.1.5 Fusarium culmorum (Fusarium head blight fungus (FHB)); 3.3 not applicable; 4.3 not applicable; 5.2.1 VIGS; 5.2.2 spray (SIGS); 6.7.1 mycotoxine production; 7.1 Concept development

Zeitschriftenaufsatz

Vadlamudi, Tharanath; Patil, Basavaprabhu L.; Kaldis, Athanasios; Sai Gopal, Divi Venkata Ramana; Mishra, Ritesh; Berbati, Margarita; Voloudakis, Andreas (2020):

DsRNA-mediated protection against two isolates of Papaya ringspot virus through topical application of dsRNA in papaya.

In: *Journal of virological methods* 275, S. 113750.

Abstract:

Papaya ringspot virus (PRSV) infections in papaya result in heavy yield losses, severely affecting the papaya industry worldwide, and hence warranting for effective control measures. In the past, transgenic papaya cultivars were developed that overexpressed parts of the PRSV genome and exhibited high levels of virus resistance. In the present study, a non-transgenic approach was employed, in which in vitro produced dsRNA molecules derived from a PRSV isolate from South India (PRSV-Tirupati) was tested for dsRNA-mediated protection against two isolates of PRSV through topical application of the dsRNA on papaya. The results showed that the dsRNA molecules from both the coat protein (CP) and helper component-proteinase (HC-Pro) genes of the PRSV-Tirupati isolate conferred 100 % resistance against PRSV-Tirupati infection. Further, the same dsRNA molecules were highly effective against the PRSV-Delhi isolate on the papaya cv. Pusa Nanha, conferring a resistance of 94 % and 81 %, respectively. Systemic papaya leaves of the dsRNA-treated plants were virus-free at 14 days post-inoculation, confirming the robustness of this non-transgenic virus control strategy. In contrast, the control TMV dsRNA did not protect against the PRSV infection. This study on the topical application of dsRNA opened up a new avenue for the control of papaya ringspot disease worldwide.

Schlagwörter:

Capsid Proteins/genetics; Carica/virology; Cysteine Endopeptidases/genetics; India; Plant Diseases/prevention & control/virology; Potyvirus/drug effects/pathogenicity; RNA, Double-Stranded/pharmacology; Viral Proteins/genetics

Kategorien:

1.3.4 Carica papaya (papaya); 2.4.4 Papaya ringspot virus (PRSV); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2 exogenous; 6.7.7 coat protein (virus); 6.7.8 infection mechanisms (virulence factors); 7.2 Concept demonstration

Zeitschriftenaufsatz

Vargas-Salinas, Mayela; Medina-Hernández, Diana; Arcos-Ortega, Guadalupe Fabiola; Luis-Villaseñor, Irasema Elizabeth; Holguín-Peña, Ramón Jaime (2021):

RNAi activation with homologous and heterologous sequences that induce resistance against the begomovirus Pepper golden mosaic virus (PepGMV).

In: *3 Biotech* 11 (3), S. 114. DOI: 10.1007/s13205-021-02653-7.

Abstract:

This study compared the transcriptional changes in Nicotiana benthamiana plants treated with homologous sequences derived from Pepper golden mosaic virus (PepGMV) and heterologous sequences that derived from another begomovirus, Tomato chino La Paz virus (ToChLPV) prior to infection by PepGMV. The results of microarray analyses identified upregulated genes associated with RNAi such as DCL2, DCL4, AGO3, AGO7, AGO10, NRPD2B (Pol IV), DRB3, CMT3, RDR6. The components that participate in different RNAi pathways were identified, including methylation induced by both constructs, as well as the code of these genes in Arabidopsis thaliana and its counterpart in N. benthamiana through different genome assembly. The expression of these genes was validated by quantitative reverse transcription polymerase chain reaction (RT-qPCR), where DCL3, DCL4, AGO1-1, AGO2, RDR6 and PPR1 showed increased expression during plant protection with the heterologous construct compared to those protected with the homologous construct. The results of this study confirmed the activation of the gene silencing mechanism at the transcriptional level with both constructs and established the possibility of their use as a protection system for both homologous and heterologous sequences.

Schlagwörter:

dsRNA; gene silencing; microarray; Tomato chino La Paz virus (ToChLPV)

Kategorien:

1.5.4 Nicotiana benthamiana; 2.4.2 Pepper golden mosaic virus (PepGMV); 3.3 not applicable; 4.3 not applicable; 5.1.1 stable transgene; 6.9 not applicable; 7.1 Concept development

Patentschrift

Venkata, Bala P. (2018):

RNAI APPROACH FOR CROP PEST PROTECTION.

Angemeldet durch DONALD DANFORTH PLANT SCIENCE CENTER [US] am 01.05.2018. Anmeldenr: MX20190012986 20180501. Veröffentlichungsnr: MX2019012986 (A). A01N63/60;A61K31/713;C12N15/113;C12N15/63. Prioriätsdaten: US201762492556P 20170501;WO2018US30506 20180501.

Abstract:

Provided herein is the identification of insect RNAi target genes (IRTG) involved in gut microbial clearance and containment and examples of a novel biotechnology for devising pesticidal RNAi approaches.

Identificación de genes blanco de ARNi de insecto (IRTG) involucrados en la depuración y la contención de los microorganismos del intestino y ejemplos de una biotecnología para concebir enfoques de ARNi plaguicida.

Kategorien:

2.2 insects

Zeitschriftenaufsatz

Vetukuri, Ramesh R.; Dubey, Mukesh; Kalyandurg, Pruthvi B.; Carlsson, Anders S.; Whisson, Stephen C.; Ortiz, Rodomiro (2021):

Spray-induced gene silencing: an innovative strategy for plant trait improvement and disease control.

In: *Crop Breed. Appl. Biotechnol.* 21 (spe), Artikel e387921S11. DOI: 10.1590/1984-70332021v21Sa24.

Abstract:

Modern plant breeding is still a time-consuming and costly process, even with the most advanced technologies such as gene editing. Hence, there is an urgent need to develop alternative means for plant trait manipulation and plant protection. RNA interference (RNAi) is a conserved cellular mechanism mediated by naturally occurring double-stranded RNA (dsRNA) and small RNAs (sRNAs) that can target mRNAs for destruction or transcript reduction. Here, we review the potential of technology based on RNAi, called spray-induced gene silencing (SIGS), as an alternative or adjunct to breeding for manipulation of endogenous gene expression in plants or pathogen control. SIGS based on exogenous application of RNA molecules in plants may be especially useful in reducing pest or pathogen impacts, thereby ameliorating biotic stresses and increasing the agronomic performance of crops.

Schlagwörter:

dsRNA; RNA, Small Interfering; RNAi (RNA interference); SIGS (spray-induced gene silencing)

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.2.2 spray (SIGS); 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Wang, Ming; Jin, Hailing (2017):

Spray-Induced Gene Silencing: a Powerful Innovative Strategy for Crop Protection.

In: *Trends in microbiology* 25 (1), S. 4–6. DOI: 10.1016/j.tim.2016.11.011.

Abstract:

Plant pathogens cause serious crop losses worldwide. Recent new studies demonstrate that spraying double-stranded RNAs (dsRNAs) and small RNAs (sRNAs) that target essential pathogen genes on plant surfaces confer efficient crop protection. This so-called spray-induced gene silencing (SIGS) strategy of disease control is potentially sustainable and environmentally friendly.

Schlagwörter:

Arabidopsis/microbiology; Crop Protection/methods; Fusarium/genetics/growth & development; Hordeum/microbiology; Plant Diseases/prevention & control; RNA Interference; RNA, Small Interfering/genetics; Sterol 14-Demethylase/genetics

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.2.2 spray (SIGS); 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Wang, Ming; Thomas, Nicholas; Jin, Hailing (2017):

Cross-kingdom RNA trafficking and environmental RNAi for powerful innovative pre- and post-harvest plant protection.

In: *Current Opinion in Plant Biology* 38, S. 133–141. DOI: 10.1016/j.pbi.2017.05.003.

Abstract:

S mall RNA (sRNA) induces RNA interference (RNAi) in almost all eukaryotes. While sRNAs can move within an organism, they can also move between interacting organisms to induce gene silencing, a phenomenon called 'cross-kingdom RNAi'. Some sRNAs from pathogens or pests move into host cells and suppress host immunity in both plants and animals; whereas some host sRNAs travel into pathogen/pest cells to inhibit their virulence. Moreover, uptake of exogenous RNAs from the environment was recently discovered in certain fungal pathogens, which makes it possible to suppress fungal diseases by directly applying pathogen-targeting RNAs on crops and post-harvest

products. This new-generation of RNA-based fungicides is powerful, environmentally friendly, and can be easily adapted to control multiple diseases simultaneously.

Schlagwörter:

Host-Pathogen Interactions/genetics/physiology; Plant Immunity/genetics/physiology; RNA, Plant/genetics; RNA, Small Interfering/genetics; Virulence/genetics/physiology

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Wang, Ming; Weiberg, Arne; Lin, Feng-Mao; Thomma, Bart P. H. J.; Huang, Hsien-Da; Jin, Hailing (2016):

Bidirectional cross-kingdom RNAi and fungal uptake of external RNAs confer plant protection.

In: *Nat. Plants* 2 (10), S. 16151. DOI: 10.1038/nplants.2016.151.

Abstract:

Aggressive fungal pathogens such as Botrytis and Verticillium spp. cause severe crop losses worldwide. We recently discovered that Botrytis cinerea delivers small RNAs (Bc-sRNAs) into plant cells to silence host immunity genes. Such sRNA effectors are mostly produced by Botrytis cinerea Dicer-like protein 1 (Bc-DCL1) and Bc-DCL2. Here we show that expressing sRNAs that target Bc-DCL1 and Bc-DCL2 in Arabidopsis and tomato silences Bc-DCL genes and attenuates fungal pathogenicity and growth, exemplifying bidirectional cross-kingdom RNAi and sRNA trafficking between plants and fungi. This strategy can be adapted to simultaneously control multiple fungal diseases. We also show that Botrytis can take up external sRNAs and double-stranded RNAs (dsRNAs). Applying sRNAs or dsRNAs that target Botrytis DCL1 and DCL2 genes on the surface of fruits, vegetables and flowers significantly inhibits grey mould disease. Such pathogen gene-targeting RNAs represent a new generation of environmentally friendly fungicides.

Schlagwörter:

Arabidopsis/genetics/immunology/microbiology; Botrytis/genetics/physiology; Plant Diseases/immunology/microbiology; Plant Immunity; RNA Interference; RNA, Fungal/genetics; RNA, Plant/genetics; Solanum lycopersicum/genetics/immunology/microbiology

Kategorien:

1.4.8 Solanum lycopersium (tomato); 1.5.3 Arabidopsis thaliana; 2.1.2 Botrytis cinerea; 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.1.3 exRNA; 4.2.2 post-transcriptional gene regulation; 5.1 endogenous; 5.1.1 stable transgene; 5.2.2 spray (SIGS); 6.5.2 RNAi machinery; 7.2 Concept demonstration

Zeitschriftenaufsatz

Wang, W. X.; Zhu, T. H.; Li, K. L.; Chen, L. F.; Lai, F. X.; Fu, Q. (2017):

Molecular characterization, expression analysis and RNAi knock-down of elongation factor 1α and 1γ from Nilaparvata lugens and its yeast-like symbiont.

In: *Bulletin of entomological research* 107 (3), S. 303–312. DOI: 10.1017/S0007485316000882.

Abstract:

In the present paper, four cDNAs encoding the alpha and gamma subunits of elongation factor 1 (EF-1) were cloned and sequenced from Nilaparvata lugens, named NlEF-1α, NlEF-1γ, and its yeast-like symbiont (YLS), named YsEF-1α and YsEF-1γ, respectively. Comparisons with sequences from other species indicated a greater conservation for EF-1α than for EF-1γ. NlEF-1α has two identical copies. The deduced amino acid sequence homology of NlEF-1α and NlEF-1γ is 96 and 64%, respectively, compared with Homalodisca vitripennis and Locusta migratoria. The deduced amino acid sequence homology of YsEF-1α and YsEF-1γ is 96 and 74%, respectively, compared with Metarhizium anisopliae and Ophiocordyceps sinensis. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis revealed that the expression level of NlEF-1α and NlEF-1γ mRNA in hemolymph, ovary, fat body and salivary glands were higher than the midgut and leg tissue. YsEF-1α and YsEF-1γ was highly expressed in fat body. The expression level of NlEF-1α was higher than that of NlEF-1γ. Through RNA interference (RNAi) of the two genes, the mortality of nymph reached 92.2% at the 11th day after treatment and the ovarian development was severely hindered. The RT-qPCR analysis verified the correlation between mortality, sterility and the down-regulation of the target genes. The expression and synthesis of vitellogenin (Vg) protein in insects injected with NlEF-1α and NlEF-1γ double-stranded RNA (dsRNA) was significantly lower than control groups. Attempts to knockdown the YsEF-1 genes in the YLS was unsuccessful. However, the phenotype of N. lugens injected with YsEF-1α dsRNA was the same as that injected with NlEF-1α dsRNA, possibly due to the high similarity (up to 71.9%) in the nucleotide sequences between NlEF-1α and YsEF-1α. We demonstrated that partial silencing of NlEF-1α and NlEF-1γ genes caused lethal and sterility effect on N. lugens. NlEF-1γ shares low identity with that of other insects and therefore it could be a potential target for RNAi-based pest management.

Schlagwörter:

Animals; DNA, Complementary/genetics/metabolism; Female; Fungal Proteins/chemistry/genetics/metabolism; gene expression; Hemiptera/genetics/growth & development/microbiology/physiology; Insect Proteins/chemistry/genetics/metabolism; Male; Nymph/genetics/growth & development/physiology; Organ Specificity; Peptide Elongation Factor 1/chemistry/genetics/metabolism; RNA Interference; Sequence Analysis, Protein; Sequence Homology, Amino Acid; Symbiosis; Yeasts/genetics/physiology

Kategorien:

1.6 not applicable; 2.2.14 Nilaparvata lugens (Brown planthopper); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2.9 microinjection; 6.1.5 translation; 7.1 Concept development

Zeitschriftenaufsatz

Werner, Bernhard Timo; Gaffar, Fatima Yousiff; Schuemann, Johannes; Biedenkopf, Dagmar; Koch, Aline Michaela (2020):

RNA-Spray-Mediated Silencing of Fusarium graminearum AGO and DCL Genes Improve Barley Disease Resistance.

In: *Frontiers in Plant Science* 11, S. 476. DOI: 10.3389/fpls.2020.00476.

Abstract:

Over the last decade, several studies have revealed the enormous potential of RNA-silencing strategies as a potential alternative to conventional pesticides for plant protection. We have previously shown that targeted gene silencing mediated by an in planta expression of non-coding inhibitory double-stranded RNAs (dsRNAs) can protect host plants against various diseases with unprecedented efficiency. In addition to the generation of RNA-silencing (RNAi) signals in planta, plants can be protected from pathogens, and pests by spray-applied RNA-based biopesticides. Despite the striking efficiency of RNA-silencing-based technologies holds for agriculture, the molecular mechanisms underlying spray-induced gene silencing (SIGS) strategies are virtually unresolved, a requirement for successful future application in the field. Based on our previous work, we predict that the molecular mechanism of SIGS is controlled by the fungal-silencing machinery. In this study, we used SIGS to compare the silencing efficiencies of computationally-designed vs. manually-designed dsRNA constructs targeting ARGONAUTE and DICER genes of Fusarium graminearum (Fg). We found that targeting key components of the fungal RNAi machinery via SIGS could protect barley leaves from Fg infection and that the manual design of dsRNAs resulted in higher gene-silencing efficiencies than the tool-based design. Moreover, our results indicate the possibility of cross-kingdom RNA silencing in the Fg-barley interaction, a phenomenon in which sRNAs operate as effector molecules to induce gene silencing between species from different kingdoms, such as a plant host and their interacting pathogens.

Schlagwörter:

AGO and DCL; Fusarium graminearium; RNA spraying; spray-induced gene silencing; virus recovery

Kategorien:

1.1.5 Hordeum vulgare (barley); 2.1.1 Fusarium graminearum; 3.3 not applicable;
4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2 exogenous; 5.2.2 spray (SIGS); 6.5.2 RNAi machinery; 7.1 Concept development

Zeitschriftenaufsatz

Willow, Jonathan; Soonvald, Liina; Sulg, Silva; Kaasik, Riina; Silva, Ana Isabel; Taning, Clauvis Nji Tizi et al. (2020):

First Evidence of Bud Feeding-Induced RNAi in a Crop Pest via Exogenous Application of dsRNA.

In: *Insects* 11 (11). DOI: 10.3390/insects11110769.

Abstract:

Spray-induced gene silencing (SIGS) is a potential strategy for agricultural pest management, whereby nucleotide sequence-specific double-stranded RNA (dsRNA) can be sprayed onto a crop; the desired effect being a consumption of dsRNA by the target pest, and subsequent gene silencing-induced mortality. Nucleotide sequence-specificity is the basis for dsRNA's perceived biosafety. A biosafe approach to pollen beetle (Brassicogethes aeneus) management in oilseed rape (Brassica napus) agroecosystems is needed. We examined the potential for SIGS in B. aeneus, via bud feeding, a field-relevant dsRNA exposure route. Oilseed rape buds were uniformly treated with dsRNA designed to target αCOP in B. aeneus. Our model control dsRNA (dsGFP) remained detectable on buds throughout the entire 3 d exposure period. When applied at 5 µg/µL, dsαCOP induced significant αCOP silencing 3 d after dietary exposure to buds treated with this dsαCOP concentration. We also observed a trend of increased αCOP silencing with increasing concentrations of dsαCOP at both 3 and 6 d. Furthermore, we observed a marginally significant and significant reduction in B. aeneus survival at 10 and 15 d, respectively. Our results suggest potential for developing a SIGS approach to B. aeneus management-though further experiments are needed to more fully understand this potential.

Schlagwörter:

biopesticide; Coleoptera; Insecticides/chemistry/pharmacology; Meligethes aeneus; Nitidulidae; rapeseed; RNAi (RNA interference)

Kategorien:

1.4.3 Brassica napus (Oilseed rape); 2.2.30 Brassicogethes aeneus (Pollen beetle); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2.2 spray (SIGS); 6.1.15 intracellular transport; 6.1.17 cell proliferation, mitosis; 7.2 Concept demonstration

Zeitschriftenaufsatz

Worrall, Elizabeth A.; Bravo-Cazar, Ana; Nilon, Alexander T.; Fletcher, Stephen J.; Robinson, Karl E.; Carr, John P.; Mitter, Neena (2019):

Exogenous Application of RNAi-Inducing Double-Stranded RNA Inhibits Aphid-Mediated Transmission of a Plant Virus.

In: *Frontiers in Plant Science* 10, S. 265. DOI: 10.3389/fpls.2019.00265.

Abstract:

Plant viruses are difficult to control, and they decrease both the quality and yield of crops, thus threatening global food security. A new approach that uses topical application of double-stranded RNA (dsRNA) to induce antiviral RNA-interference has been shown to be effective at preventing virus infection in a range of plants following mechanical inoculation. In this study, topical application of dsRNA was effective against mechanical inoculation and aphid-mediated inoculation with the potyvirus bean common mosaic virus (BCMV). Topical application of dsRNAs targeting either the coding region of the potyviral nuclear inclusion b (NIb) protein (BCMVNIb-dsRNA) or the coat protein (CP) coding region (BCMVCP-dsRNA) protected Nicotiana benthamiana and cowpea (Vigna unguiculata) plants against mechanical inoculation with BCMV. BCMVCP-dsRNA was selected for subsequent aphid transmission experiments. BCMVCP-dsRNA was loaded onto layered double hydroxide nanoparticles to form BCMVCP-BioClay which is a more stable formulation for delivering dsRNA than naked dsRNA. BCMVCP-BioClay was shown to be successful in protecting plants against BCMV transmission by the aphid Myzus persicae. Spraying detached N. benthamiana leaves with BCMVCP-BioClay 5 days prior to exposure to viruliferous aphids protected the leaves from infection by BCMV. Importantly, spraying of intact N. benthamiana and cowpea plants with BCMVCP-BioClay 5 days prior to exposure to viruliferous aphids protected plants of both species from BCMV infection. This study demonstrates that topical application of dsRNA using BioClay protects plants from aphid-mediated virus transmission, which is an important first step toward developing practical application of this approach in crop protection.

Kategorien:

1.4.10 Vigna unguiculata; 1.5.4 Nicotiana benthamiana; 2.4.6 Potyvirus bean common mosaic virus (BCMV); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2 exogenous; 6.7 Pathogenesis; 7.2 Concept demonstration

Zeitschriftenaufsatz

Wytinck, Nick; Manchur, Christopher L.; Li, Vivian H.; Whyard, Steve; Belmonte, Mark F. (2020):

dsRNA Uptake in Plant Pests and Pathogens: Insights into RNAi-Based Insect and Fungal Control Technology.

In: *Plants (Basel, Switzerland)* 9 (12). DOI: 10.3390/plants9121780.

Abstract:

Efforts to develop more environmentally friendly alternatives to traditional broad-spectrum pesticides in agriculture have recently turned to RNA interference (RNAi) technology. With the built-in, sequence-specific knockdown of gene targets following delivery of double-stranded RNA (dsRNA), RNAi offers the promise of controlling pests and pathogens without adversely affecting non-target species. Significant advances in the efficacy of this technology have been observed in a wide range of species, including many insect pests and fungal pathogens. Two different dsRNA application methods are being developed. First, host induced gene silencing (HIGS) harnesses dsRNA production through the thoughtful and precise engineering of transgenic plants and second, spray induced gene silencing (SIGS) that uses surface applications of a topically applied dsRNA molecule. Regardless of the dsRNA delivery method, one aspect that is critical to the success of RNAi is the ability of the target organism to internalize the dsRNA and take advantage of the host RNAi cellular machinery. The efficiency of dsRNA uptake mechanisms varies across species, and in some uptake is negligible, rendering them effectively resistant to this new generation of control technologies. If RNAi-based methods of control are to be used widely, it is critically important to understand the mechanisms underpinning dsRNA uptake. Understanding dsRNA uptake mechanisms will also provide insight into the design and formulation of dsRNAs for improved delivery and provide clues into the development of potential host resistance to these technologies.

Schlagwörter:

clathrin-mediated endocytosis; dsRNA; dsRNA uptake; fungi; insects; plant protection; RNAi (RNA interference); SID proteins; SIGS (spray-induced gene silencing)

Kategorien:

1.6 not applicable; 2.1 fungi; 2.2 insects; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Xu, Xiang; Yu, Tingting; Zhang, Daoshun; Song, Hongping; Huang, Kun; Wang, Yong et al. (2023):

Evaluation of the anti-viral efficacy of three different dsRNA nanoparticles against potato virus Y using various delivery methods.

In: *Ecotoxicology and environmental safety* 255, S. 114775. DOI: 10.1016/j.ecoenv.2023.114775.

Abstract:

Nanoparticles (NPs) derived from RNA interference (RNAi) are considered a potentially revolutionary technique in the field of plant protection in the future. However, the application of NPs in RNAi is hindered by the conflict between the high cost of RNA production and the large quantity of materials required for field application. This study aimed to evaluate the antiviral efficacy of commercially available nanomaterials, such as chitosan quaternary ammonium salt (CQAS), amine functionalized silica nano powder (ASNP), and carbon quantum dots (CQD), that carried double-stranded RNA (dsRNA) via various delivery methods, including infiltration, spraying, and root soaking. ASNP-dsRNA NPs are recommended for root soaking, which is considered the most effective method of antiviral compound application. The most effective antiviral compound tested was CQAS-dsRNA NPs delivered by root soaking. Using fluorescence, FITC-CQAS-dsCP-Cy3, and CQD-dsCP-Cy3 NPs demonstrated the uptake and transport pathways of dsRNA NPs in plants when applied to plants in different modes. The duration of protection with NPs applied in various modes was then compared, providing references for evaluating the retention period of various types of NPs. All three types of NPs effectively silenced genes in plants and afforded at least 14 days of protection against viral infection. Particularly, CQD-dsRNA NPs could protect systemic leaves for 21 days following spraying.

Schlagwörter:

Antiviral Agents/pharmacology; Nanoparticles; Potyvirus/genetics; RNA Interference; RNA, Double-Stranded

Kategorien:

1.5.4 Nicotiana benthamiana; 2.4.5 Potato virus Y (PVY); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2.4.1 nanoparticle-mediated dsRNA; 6.9 not applicable; 7.1 Concept development

Zeitschriftenaufsatz

Yan, Shuo; Ren, Binyuan; Zeng, Bo; Shen, Jie (2020):

Improving RNAi efficiency for pest control in crop species.

In: *BioTechniques* 68 (5), S. 283–290. DOI: 10.2144/btn-2019-0171.

Abstract:

The application of RNAi promotes the development of novel approaches toward plant protection in a sustainable way. Genetically modified crops expressing dsRNA have been developed as commercial products with great potential in insect pest management. Alternatively, some nontransformative approaches, including foliar spray, irrigation and trunk injection, are favorable in actual utilization. In this review, we summarize the recent progress and successful cases of RNAi-based pest management strategy, explore essential implications and possibilities to improve RNAi efficiency by delivery of dsRNA through transformative and nontransformative approaches, and highlight the remaining challenges and important issues related to the application of this technology.

Schlagwörter:

Crops, Agricultural/genetics; dsRNA delivery; foliar spray; nanoparticle; nontransformative RNAi product; Pest Control/methods; Plants, Genetically Modified/genetics; RNA Interference; RNAi efficiency; RNAi-based pest management; transformative RNAi product; transplastomic crop

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Yan, Shuo; Ren, Bin-Yuan; Shen, Jie (2021):

Nanoparticle-mediated double-stranded RNA delivery system: A promising approach for sustainable pest management.

In: *Insect science* 28 (1), S. 21–34. DOI: 10.1111/1744-7917.12822.

Abstract:

RNA interference (RNAi) targeting lethal genes in insects has great potential for sustainable crop protection. Compared with traditional double-stranded (ds)RNA delivery systems, nanoparticles such as chitosan, liposomes, and cationic dendrimers offer advantages in delivering dsRNA/small interfering (si)RNA to improve RNAi efficiency, thus promoting the development and practice of RNAi-based pest management strategies. Here, we illustrate the limitations of traditional dsRNA delivery systems, reveal the mechanism of nanoparticle-mediated RNAi, summarize the recent progress and successful applications of nanoparticle-mediated RNAi in pest management, and finally address the prospects of nanoparticle-based RNA pesticides.

Schlagwörter:

Animals; Insect Control/methods; Insecta; Nanoparticles/analysis; RNA Interference; RNA, Double-Stranded/administration & dosage; RNA, Small Interfering/analysis

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.2.4.1 nanoparticle-mediated dsRNA; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Yan, Shuo; Shen, Jie (2022):

Application of Nanoparticle-Mediated RNAi for Efficient Gene Silencing and Pest Control on Soybean Aphids.

In: *Methods in molecular biology (Clifton, N.J.)* 2360, S. 307–315. DOI: 10.1007/978-1-0716-1633-8_22.

Abstract:

The application of the RNA interference (RNAi) mechanism promotes the development of novel approaches toward sustainable crop protection. Compared with traditional double-stranded (ds)RNA delivery systems, nanoparticles offer great advantages in delivering dsRNA to improve RNAi efficiency, thus promoting the development and practice of RNAi-based pest management strategies. Here, we described a transdermal dsRNA delivery system with a nanosized star polycation, and presented a method to improve RNAi efficiency to increase the control effect against aphids. Insect gene functional analysis and pest management can be achieved by this method.

Schlagwörter:

Animals; Aphids/genetics; gene silencing; Nanoparticles; Pest Control; RNA Interference; RNA, Double-Stranded/genetics; Soybeans

Kategorien:

1.6 not applicable; 2.2.21 Soy bean aphid; 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2.4.1 nanoparticle-mediated dsRNA; 6.9 not applicable; 7.1 Concept development

Zeitschriftenaufsatz

Yu, Hai-Yan; Zhou, Zhi-Feng; Jia, Jun-Qiang; Gui, Zhong-Zheng (2016):

Cloning, expression and functional analysis of a delta 6-desaturase gene from the silkworm, Bombyx mori L.

In: *Journal of Asia-Pacific Entomology* 19 (3), S. 581–587. DOI: 10.1016/j.aspen.2016.05.002.

Abstract:

Delta 6-fatty acid desaturase is a membrane-bound enzyme, which is the rate-limiting factor in the biosynthesis of polyunsaturated fatty acids. In this study, a novel delta 6-desaturase gene was cloned from Bombyx mori (BmD6DES). Sequencing analysis revealed that BmD6DES has an open reading frame of 1357 by that encodes 448 amino acids. Heterologous expression in yeast demonstrated that BmD6DES could synthesize gamma-linolenic acid (GLA, 18:3, Delta(6.9.12)) by utilizing the endogenous substrate linoleic acid (LA, 18:2, Delta(9.12)). We found that BmD6DES transcripts were distributed in almost all B. mori tissues, with high expression levels observed at the 5th instar larval, pupal, and adult moth stages. A functional analysis of BmD6DES was performed by measuring mRNA levels after temperature stress, fungal infection, and RNA interference (RNAi). The results indicated that the highest expression of BmD6DES was observed at low temperatures (0 degrees C) and 6 h to 36 h after fungal infection. qPCR analysis demonstrated that BmD6DES mRNA levels in pupa after BmD6DES RNAi treatment were significantly reduced from 12 h to 72 h compared to those in the control group. Our findings suggest that BmD6DES not only induces the formation of the third carbon-carbon double bond in the LA carbon chain, but also leads to sensitivity to low-temperature stress and fungal infection. These results imply that BmD6DES is a key gene in the gamma-linolenic acid pathway during B. mori development.

Schlagwörter:

bollworm (helicoverpa armigera); Bombyx; Cloning, Molecular; functional analysis; gene expression

Kategorien:

1.6 not applicable; 2.2.20 Bombyx mori (Silkworm); 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.3.2 fatty acids synthesis; 7.1 Concept development

Zeitschriftenaufsatz

Yu, Xiu-Dao; Liu, Zong-Cai; Huang, Si-Liang; Chen, Zhi-Qin; Sun, Yong-Wei; Duan, Peng-Fei et al. (2016):

RNAi-mediated plant protection against aphids.

In: *Pest management science* 72 (6), S. 1090–1098. DOI: 10.1002/ps.4258.

Abstract:

Aphids (Aphididae) are major agricultural pests that cause significant yield losses of crop plants each year by inflicting damage both through the direct effects of feeding and by vectoring harmful plant viruses. Expression of double-stranded RNA (dsRNA) directed against suitable insect target genes in transgenic plants has been shown to give protection against pests through plant-mediated RNA interference (RNAi). Thus, as a potential alternative and effective strategy for insect pest management in agricultural practice, plant-mediated RNAi for aphid control has received close attention in recent years. In this review, the mechanism of RNAi in insects and the so far explored effective RNAi target genes in aphids, their potential applications in the development of transgenic plants for aphid control and the major challenges in this regard are reviewed, and the future prospects of using plant-mediated RNAi for aphid control are discussed. This review is intended to be a helpful insight into the generation of aphid-resistant plants through plant-mediated RNAi strategy.

Schlagwörter:

Animals; aphid control; Aphids; Crops, Agricultural/genetics/parasitology; dsRNA; Insect Control/methods; plant; Plants, Genetically Modified/genetics/parasitology; RNA Interference; RNAi (RNA interference)

Kategorien:

1.6 not applicable; 2.2.38 aphids; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Zeng, Jun; Gupta, Vijai Kumar; Jiang, Yueming; Yang, Bao; Gong, Liang; Zhu, Hong (2019):

Cross-Kingdom Small RNAs Among Animals, Plants and Microbes.

In: *Cells* 8 (4). DOI: 10.3390/cells8040371.

Abstract:

Small RNAs (sRNAs), a class of regulatory non-coding RNAs around 20~30-nt long, including small interfering RNAs (siRNAs) and microRNAs (miRNAs), are critical regulators of gene expression. Recently, accumulating evidence indicates that sRNAs can be transferred not only within cells and tissues of individual organisms, but also across different eukaryotic species, serving as a bond connecting the animal, plant, and microbial worlds. In this review, we summarize the results from recent studies on cross-kingdom sRNA communication. We not only review the horizontal transfer of sRNAs among animals, plants and microbes, but also discuss the mechanism of RNA interference (RNAi) signal transmission via cross-kingdom sRNAs. We also compare the advantages of host-induced gene silencing (HIGS) and spray-induced gene silencing (SIGS) technology and look forward to their applicable prospects in controlling fungal diseases.

Schlagwörter:

Animals; Bacteria/genetics; Gene Expression Regulation/genetics; gene silencing; Gene Transfer, Horizontal/genetics/physiology; Humans; MicroRNAs/genetics/metabolism; Plants/genetics; RNA, Bacterial/genetics; RNA, Plant/genetics; RNA, Small Interfering/genetics; RNA, Small Untranslated/genetics/metabolism

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Zhang, Jiang; Khan, Sher Afzal; Hasse, Claudia; Ruf, Stephanie; Heckel, David G.; Bock, Ralph (2015):

Pest control. Full crop protection from an insect pest by expression of long double-stranded RNAs in plastids.

In: *Science (New York, N.Y.)* 347 (6225), S. 991–994. DOI: 10.1126/science.1261680.

Abstract:

Double-stranded RNAs (dsRNAs) targeted against essential genes can trigger a lethal RNA interference (RNAi) response in insect pests. The application of this concept in plant protection is hampered by the presence of an endogenous plant RNAi pathway that processes dsRNAs into short interfering RNAs. We found that long dsRNAs can be stably produced in chloroplasts, a cellular

compartment that appears to lack an RNAi machinery. When expressed from the chloroplast genome, dsRNAs accumulated to as much as 0.4% of the total cellular RNA. Transplastomic potato plants producing dsRNAs targeted against the β-actin gene of the Colorado potato beetle, a notorious agricultural pest, were protected from herbivory and were lethal to its larvae. Thus, chloroplast expression of long dsRNAs can provide crop protection without chemical pesticides.

Schlagwörter:

Actins/antagonists & inhibitors/genetics; Animals; Coleoptera/genetics/pathogenicity; Crops, Agricultural/genetics/parasitology; Genetic Vectors; Pest Control, Biological/methods; Plant Leaves/genetics/parasitology; Plastids/genetics; RNA Interference; RNA, Double-Stranded/genetics; RNA, Small Interfering/genetics/metabolism; Solanum tuberosum/genetics/parasitology; Transformation, Genetic

Kategorien:

1.4.5 Solanum tuberosum (potato); 2.2.19 Leptinotarsa decemlineata (Colorado potato beetle); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.1.1 stable transgene; 5.1.1.1 production in chloroplasts; 6.1.12 cytosceleton; 7.2 Concept demonstration

Zeitschriftenaufsatz

Zhang, Yashu; Zhao, Xiao; Ji, Jinyun; Kang, Tinghao; Li, Jianhong; Wan, Hu (2016):

Molecular cloning and characterization of a phenylalanine hydroxylase from the common cutworm Spodoptera litura.

In: *Journal of Asia-Pacific Entomology* 19 (2), S. 365–370. DOI: 10.1016/j.aspen.2016.04.005.

Abstract:

In the present study, a phenylalanine hydroxylase (PAH) was cloned and characterized from the common cutworm Spodoptera litura (SIPAH). The SIPAH gene is an 1863 bp full-length cDNA with a 1371 bp open reading frame that encodes a predicted 52.35 kDa protein of 457 amino acid residues with an isoelectric point of 5.39. The SIPAH gene contains 6 exons and 5 introns. A multiple sequence analysis of SIPAH showed an 88-68% sequence identity to Papilio xuthus PAH and other insect PAHs. A phylogenetic tree revealed that SIPAH is a novel member of the PAH family. Tissue distribution analyses indicated that SIPAH is constitutively expressed in the epidermis, midgut, and fat body, with the highest expression occurring in the fat body on days 2 and 3 of the sixth-instar larval stage and day 1 of the prepupa stage. However, RNAi-mediated SIPAH gene silencing had no observable phenotype in S. litura larvae. Additionally, SIPAH expression is acutely up-regulated in the fat body after injection with Bacillus thuringiensis, Escherichia coli, or Beauveria bassiana. Therefore, these results provide a molecular basis for the functional roles of SIPAH during development and the innate immune response in S. litura.

Schlagwörter:

common cutworm; dsRNA; insect immunity; phenylalanine hydroxylase; Spodoptera litura

Kategorien:

1.6 not applicable; 2.2.28 Spodoptera litura (Common cutworm); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2.1 mRNA degradation; 5.3 not applicable; 6.1.5 translation; 7.1 Concept development

Zeitschriftenaufsatz

Zhang, Yifan; Deng, Fei; Fan, Yongliang; Zhao, Zhangwu (2016):

Effects of carboxylesterase gene silence on wheat aphid Sitobion avenae (Fabricius).

In: *Journal of Asia-Pacific Entomology* 19 (2), S. 341–345. DOI: 10.1016/j.aspen.2016.03.011.

Abstract:

Multifunctional carboxylesterase (CarE) has been found in all animals, plants and microbes, and belongs to a superfamily enzyme of serine hydrolase involved in detoxification, allelochemical tolerance and some specific hormone or pheromone metabolism. Insects usually utilize carboxylesterases to detoxify xenobiotics, and positively correlated with insect resistance to some insecticides. Despite the importance of CarEs in insects, carboxylesterases and their functions in wheat aphid Sitobion avenae (Fabricius) have not been clear. In this study, a sequence that encodes a carboxylesterase protein from S. avenae (SaCarE) was sequenced and cloned. After aligning the encoded amino acid sequence of the SaCarE gene with other known CarEs of insects, we found that the CarE gene was highly conserved in insects. The SaCarE mRNA levels at different developmental stages of S. avenue were gradually increased from the first instar of nymphs to adult stage. RNAi was employed to further explore its functions, in which oral ingestion of SaCarE double-stranded RNA from the third instar nymph significantly knocked down SaCarE expression, and significantly decreased ecdysis index in S. avenae. These results indicate that SaCarE is functional in S. avenae and could serve one of the potential target genes for management of S. avenae. (C) 2016 Korean Society of Applied Entomology, Taiwan Entomological Society and Malaysian Plant Protection Society. Published by Elsevier B.V.

Schlagwörter:

carboxylesterase; RNAi (RNA interference); Sitobion avenae; wheat aphid

Kategorien:

1.6 not applicable; 2.2.29 Sitobion avenae (Wheat aphid); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2.1 mRNA degradation; 5.3 not applicable; 6.4.3 pheromon production/mating behaviour; 7.1 Concept development

Zeitschriftenaufsatz

Zhao, Jian-Hua; Zhang, Tao; Liu, Qing-Yan; Guo, Hui-Shan (2021):

Trans-kingdom RNAs and their fates in recipient cells: advances, utilization, and perspectives.

In: *Plant Communications* 2 (2), S. 100167. DOI: 10.1016/j.xplc.2021.100167.

Abstract:

The phenomenon and potential mechanisms of trans-kingdom RNA silencing (or RNA interference, RNAi) are among the most exciting topics in science today. Based on trans-kingdom RNAi, host-induced gene silencing (HIGS) has been widely applied to create crops with resistance to various pests and pathogens, overcoming the limitations of resistant cultivars. However, a lack of transformation technology in many crops limits the application of HIGS. Here, we describe the various fates of trans-kingdom RNAs in recipient organisms. Based on the assumption that small RNAs can be transferred between the host and its microbiome or among microbiome members, we propose a possible alternative strategy for plant protection against pathogens without the need for crop genetic modification.

Schlagwörter:

Microbiota; Plant Cells/metabolism; RNA Interference; RNA, Bacterial/genetics; RNA, Fungal/analysis; RNA, Plant/genetics

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.1 endogenous; 6.9 not applicable; 7.3 not applicable

Zeitschriftenaufsatz

Zheng, Wenping; Liu, Yaru; Zheng, Weiwei; Xiao, Yunli; Zhang, Hongyu (2015):

Influence of the silencing sex-peptide receptor on Bactrocera dorsalis adults and offspring by feeding with ds-spr.

In: *Journal of Asia-Pacific Entomology* 18 (3), S. 477–481. DOI: 10.1016/j.aspen.2015.05.004.

Abstract:

RNA interference (RNAi) has been shown to control insect pests using plant dsRNA expression. A key question in potentially applying RNAi is the possible effects on insects after being fed with dsRNA produced by a transgenic plant. Sex peptide receptor (spr) is the key gene that regulates the suppression of female receptivity and induction of oviposition. In this study, the expression level of the spr gene was significantly down-regulated to 52% by feeding Bactrocera dorsalis adults with ds-spr. The RNAi effects of continuous feeding ds-spr to adults led to a highly mortality rate, decreased

their egg production capacity and profoundly impacted the eclosion rate of their offspring. Our results demonstrate that RNAi through uninterrupted dsRNA feeding can be used as a strategy to control insect pests. Moreover, the research presented here provides a potential RNAi target gene for controlling B. dorsalis and a theoretical basis for universally applying RNAi in insect pest management.

Schlagwörter:

Bactrocera dorsalis; continuous feeding; RNA Interference; spr

Kategorien:

1.6 not applicable; 2.2.15 Bactrocera dorsalis (Oriental fruit fly); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2.1 mRNA degradation; 5.3 not applicable; 6.4.1 egg development in females; 7.1 Concept development

Zeitschriftenaufsatz

Zhu, Bin; Shan, Jinqiong; Li, Ran; Liang, Pei; Gao, Xiwu (2019):

Identification and RNAi-based function analysis of chitinase family genes in diamondback moth, Plutella xylostella.

In: *Pest management science* 75 (7), S. 1951–1961. DOI: 10.1002/ps.5308.

Abstract:

BACKGROUND

Insect chitinases play a vital part in chitin degradation in exoskeletons and gut linings during the molting process, and therefore are considered potential targets for new insecticide designs or RNA interference (RNAi)-based pest management. Systematic functional analysis of chitinase genes has already been conducted in several insect pests, but not Plutella xylostella.

RESULTS

In this study, 13 full-length chitinase transcripts were obtained in P. xylostella. Developmental and tissue-specific expression pattern analysis revealed that seven chitinase transcripts were periodically expressed during molting stage and mainly expressed in the integument or midgut, including PxCht3, PxCht5, PxCht6-2, PxCht7, PxCht8, PxCht10 and PxCht-h. RNAi-mediated knockdown of these specific expressed genes revealed that PxCht5 and PxCht10 were essential in larval molting, pupation and eclosion, and PxCht7 was indispensable only in eclosion. No significant effects were observed on insect survival or normal development when the rest chitinase transcripts were suppressed by RNAi.

CONCLUSION

Our results indicated the function of P. xylostella chitinase family genes during the molting process, and may provide potential targets for RNAi-based management of P. xylostella. © 2018 Society of Chemical Industry.

Schlagwörter:

Animals; Chitinases/genetics; Gastrointestinal Tract/enzymology/growth & development/metabolism; Gene Expression Regulation, Developmental; Integumentary

System/growth & development; Molting/genetics; Moths/enzymology/genetics/growth & development; RNA Interference

Kategorien:

1.6 not applicable; 2.2.11 Plutella xylostella (Diamondback moth); 3.3 not applicable; 4.1.2 intermolecular dsRNA; 4.2.2 post-transcriptional gene regulation; 5.2.9 microinjection; 6.2.3 chitin synthesis; 6.2.4 molting; 6.2.5.1 metamorphosis; 7.1 Concept development

Zeitschriftenaufsatz

Zotti, Moises; Dos Santos, Ericmar Avila; Cagliari, Deise; Christiaens, Olivier; Taning, Clauvis Nji Tizi; Smagghe, Guy (2018):

RNA interference technology in crop protection against arthropod pests, pathogens and nematodes.

In: *Pest management science* 74 (6), S. 1239–1250. DOI: 10.1002/ps.4813.

Abstract:

Scientists have made significant progress in understanding and unraveling several aspects of double-stranded RNA (dsRNA)-mediated gene silencing during the last two decades. Now that the RNA interference (RNAi) mechanism is well understood, it is time to consider how to apply the acquired knowledge to agriculture and crop protection. Some RNAi-based products are already available for farmers and more are expected to reach the market soon. Tailor-made dsRNA as an active ingredient for biopesticide formulations is considered a raw material that can be used for diverse purposes, from pest control and bee protection against viruses to pesticide resistance management. The RNAi mechanism works at the messenger RNA (mRNA) level, exploiting a sequence-dependent mode of action, which makes it unique in potency and selectivity compared with conventional agrochemicals. Furthermore, the use of RNAi in crop protection can be achieved by employing plant-incorporated protectants through plant transformation, but also by non-transformative strategies such as the use of formulations of sprayable RNAs as direct control agents, resistance factor repressors or developmental disruptors. In this review, RNAi is presented in an agricultural context (discussing products that have been launched on the market or will soon be available), and we go beyond the classical presentation of successful examples of RNAi in pest-insect control and comprehensively explore its potential for the control of plant pathogens, nematodes and mites, and to fight against diseases and parasites in beneficial insects. Moreover, we also discuss its use as a repressor for the management of pesticide-resistant weeds and insects. Finally, this review reports on the advances in non-transformative dsRNA delivery and the production costs of dsRNA, and discusses environmental considerations. © 2017 Society of Chemical Industry.

Schlagwörter:

Animals; Arthropods; bacteria; Crop Protection/methods; fungi; Nematoda; Pest Control/methods; RNA Interference; RNA, Double-Stranded/genetics

Kategorien:

1.6 not applicable; 2.7 not applicable; 3.3 not applicable; 4.3 not applicable; 5.3 not applicable; 6.9 not applicable; 7.3 not applicable