

Host-Induced Gene Silencing: A Novel Strategy in Vegetable Crop Protection

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Abstract

Host-Induced Gene Silencing (HIGS) leverages the plant's own gene silencing machinery to target genes of invading pests and pathogens. By genetically engineering vegetable crops to produce double-stranded RNAs (dsRNAs) or hairpin RNAs matching essential pest genes, HIGS triggers RNA interference (RNAi) across species boundaries. This approach has shown promise in multiple vegetable families. For example, transgenic tomatoes expressing dsRNA against insect genes (such as *Tuta absoluta* V-ATPase-A) or fungal genes (such as *Fusarium oxysporum* virulence factors) exhibit significantly reduced damage. Similarly, cucurbit plants silencing viral coat protein genes (e.g. ZYMV CP) become resistant to common viruses. Recent trials in brassicas and legumes have targeted clubroot effectors and aphid genes, respectively, achieving measurable resistance. Despite these successes, HIGS faces regulatory and social considerations typical of genetically modified solutions. This article reviews the mechanisms of HIGS, highlights examples across Solanaceae, Cucurbitaceae, Brassicaceae, and Fabaceae crops, and discusses regulatory and acceptance issues. The combined evidence suggests HIGS could become a durable, species-specific complement to traditional plant breeding and chemical control methods.

Keywords: Host-induced gene silencing; RNA interference; vegetable crops; pest resistance; biotechnology; genetic engineering; regulatory issues.

Introduction

Vegetable crops worldwide suffer major losses from pests and diseases. Insects (like aphids, whiteflies, and caterpillars), fungal and oomycete pathogens (such as *Fusarium*, *Verticillium*, and *Phytophthora*), nematodes (root-knot nematodes), and viruses (e.g. mosaic and yellowing viruses) all reduce yields and quality. Conventional control often relies on chemical pesticides or breeding for resistance, but these approaches have limitations.

Pesticides can harm the environment and human health, and pests may evolve resistance. Breeding for resistance can be slow and may not keep pace with rapidly evolving pathogens. In this context, RNA interference (RNAi) has emerged as a novel biotechnological strategy to directly silence critical pest genes. Host-Induced Gene Silencing (HIGS) is an RNAi-based strategy in which a plant is engineered to produce double-stranded RNA (dsRNA) molecules matching a target gene of the pest or pathogen. When the pest feeds on or infects the plant, it takes up these dsRNAs or processed small RNAs, which trigger the RNAi pathway in the pest. The pest's own mRNA that matches the dsRNA is cleaved and degraded, effectively silencing the gene. Importantly, HIGS uses the plant as a vector to deliver gene-specific RNAi against the invader. This approach can be highly specific (targeting only the pest gene) and does not involve systemic chemicals. The concept of HIGS has been demonstrated in many systems. Early work showed that barley producing dsRNA against a fungal powdery mildew gene reduced infection, and since then over a hundred examples have been reported in cereals, legumes, and vegetables. In vegetables, applications span insect control (e.g. targeting digestive or chitin genes in caterpillars and aphids), nematode suppression (by silencing nematode effectors), fungal disease resistance (silencing pathogen virulence genes), and viral resistance (silencing viral replicases or coat proteins).

Mechanism of Host-Induced Gene Silencing

1. HIGS exploits the natural RNAi machinery shared by eukaryotes. In plants, Dicer-like enzymes cleave dsRNA into small interfering RNAs (siRNAs), which are loaded into an Argonaute-containing RNA-induced silencing complex (RISC). If these siRNAs complement a pest or pathogen RNA, the RISC guides cleavage of the invader RNA, silencing the gene. In HIGS, the plant is engineered to produce dsRNA matching a pest gene. During infection or feeding, siRNAs (or longer dsRNAs) are transported into the pathogen or pest cells. The invaded organism's own RNAi pathway then amplifies the signal and degrades the matching mRNA.
2. Several features of HIGS are important. It is cross-kingdom: plant-derived RNAs affect fungi, nematodes, insects, and even viruses and viroids. It is sequence-specific: only genes with sufficient homology to the dsRNA are silenced. It often targets essential genes such as those for feeding, development, or pathogenesis. For example, delivering dsRNA that matches a pest insect's *vacuolar ATPase* or *trehalose synthase* genes can be lethal to the insect, as those genes are vital for survival. Similarly, silencing a fungal pathogen's effector or toxin-synthesis gene can reduce its ability to infect. HIGS constructs are usually expressed as inverted-repeat hairpin RNA

(hpRNA) under a constitutive or tissue-specific promoter, resulting in high levels of dsRNA in the plant tissue. In some systems (e.g. potato for Colorado potato beetle), the dsRNA has even been expressed in plastids to avoid processing by plant Dicers and thus maximize uptake by pests.

3. Research over the last decade confirms that HIGS-derived RNAs can move from plant to pathogen. For instance, in cotton engineered with a HIGS construct targeting *Verticillium dahliae*, siRNAs were detected inside the fungus and the target gene was silenced in the pathogen. In another study, *Arabidopsis* expressing dsRNA against a *Verticillium* gene caused the fungus to lose virulence. These results indicate that host-delivered siRNAs can cross cell boundaries and trigger long-lasting trans-kingdom gene silencing, even in plant vascular-colonizing pathogens.
4. Together, the mechanisms underlying HIGS – plant production of dsRNA, inter-kingdom RNA trafficking, and sequence-specific RNAi in the invader – form a powerful framework for crop protection. The key is identifying suitable target genes in the pest/pathogen and achieving sufficient RNA expression in the host. The next sections survey how this has been done in different vegetable crop groups.

HIGS in Solanaceous Vegetable Crops

- The Solanaceae family (tomato, potato, pepper, eggplant, tobacco, etc.) includes many economically important vegetables. These crops face diverse threats: insects like the tomato leafminer (*Tuta absoluta*) and Colorado potato beetle, fungal diseases like Fusarium and Verticillium wilts, oomycete blights, and viruses such as Potato virus Y (PVY). HIGS has been explored for many of these.
- One of the first and most striking examples is the transgenic tomato targeting *Tuta absoluta*. In a PeerJ study, tomato plants were transformed with hairpins against *Tuta absoluta* V-ATPase-A and arginine kinase genes, both critical for the insect's physiology. These transgenic tomatoes showed up to a 60% reduction in *Tuta* gene transcripts, markedly higher larval mortality, and significantly less leaf feeding damage compared to wild-type plants. This demonstrates that silencing a pest's own genes from within the host can suppress pest outbreaks on tomato. Other insects have been targeted similarly. For example, plastid-transformed potato plants were engineered to produce 200 bp dsRNA against the Colorado potato beetle β -actin gene. The result was very high larval mortality and severe growth inhibition in the beetles.

These insect-targeting HIGS cases in solanaceous plants illustrate the principle that essential insect genes can be knocked down by plant-delivered RNA.

- Apart from insects, fungi and oomycetes have been tackled via HIGS in Solanaceae. Fusarium wilt is a common problem in tomato. A recent 2024 study reported transgenic tomato lines expressing dsRNA hairpins for three *Fusarium oxysporum* specific-fasciclin-like protein genes (FoFLP1, FoFLP4, FoFLP5). These FoFLP proteins are involved in fungal adhesion and colonization. The RNAi tomato lines showed a significant reduction in fungal transcripts for these genes, delayed onset of wilt symptoms, and lower root colonization by *Fusarium* compared to control plants. The HIGS plants suffered much less wilt disease, confirming that silencing multiple pathogen genes can protect tomatoes. In the same vein, Verticillium wilt – caused by *Verticillium dahliae* – has been mitigated by HIGS. Transgenic tomato expressing hairpins for *V. dahliae* virulence genes (such as VdH1) displayed much lower disease severity, showing that vascular wilt pathogens too can be controlled by host-delivered RNA silencing.
- A landmark example in potato (another Solanaceae) is HIGS against late blight (caused by the oomycete *Phytophthora infestans*). Researchers transferred a hairpin construct against the *P. infestans* acetolactate synthase (ALS) gene into potato. The ALS enzyme is key for amino acid synthesis. The resulting transgenic lines showed efficient silencing of the pathogen ALS gene during infection (evidenced by a reporter assay and qRT-PCR). Most importantly, these HIGS potatoes had dramatically enhanced late-blight resistance in controlled assays and even in field trials in Europe and the USA. This is one of the clearest demonstrations that HIGS can work under realistic agricultural conditions.
- Other solanaceous crops are also under exploration. Tobacco, while not a vegetable crop, serves as a model. Tobacco plants producing dsRNA against *Bemisia tabaci* (whitefly) trehalose-6-phosphate synthase genes (BtTPS1, BtTPS2) caused ~90% whitefly adult mortality and reduced nymph survival. This suggests the approach could translate to tomato and pepper, which suffer whitefly infestations and whitefly-vectored viruses. Similarly, targeting aphids has been demonstrated: transgenic potato lines expressing dsRNA against the green peach aphid (*Myzus persicae*) *Macrophage Inhibitory Factor 1* (MIF1) gene caused about 65–77% aphid mortality on leaves. Since *M. persicae* also attacks tomato and potato in the field, this result offers a potential strategy for reducing aphid damage to solanaceous vegetables.

- In summary, Solanaceae-targeted HIGS research has covered many pest types. Insects and nematodes have been controlled by silencing their vital genes (e.g. *V-ATPase*, *trehalose synthase*, *actin*, *MIF1*) from within the host plant. Fungal and oomycete pathogens have been controlled by silencing pathogenicity or metabolic genes (e.g. *Fusarium* FLPs, *Verticillium* effectors, *Phytophthora* ALS). The collected evidence suggests that HIGS constructs can be designed for tomato, potato, pepper and related vegetables with multiple target genes in pests.

HIGS in Cucurbitaceae Vegetable Crops

1. The Cucurbitaceae family includes cucumber, melon, squash, pumpkin and gourds. Key pests include viruses (Zucchini yellow mosaic virus, Cucumber mosaic virus, etc.), fungal diseases (powdery mildew, downy mildew), and insects (whiteflies, aphids, and certain moth larvae). Many cucurbits lack strong natural resistance to these threats, making HIGS an attractive tool.
2. To date, most HIGS work in cucurbits has focused on viral diseases. Cucurbit crops are particularly prone to potyviruses and mosaic viruses. Transgenic melon plants expressing antisense RNA complementary to the coat protein gene of Zucchini yellow mosaic virus (ZYMV) showed robust resistance: the virus was unable to establish infection and cause mosaic symptoms in these plants. In another study, squash and cucumber expressing hairpin RNA against the Papaya ringspot virus (PRSV) coat protein (type W strain) were protected against that virus. Similarly, virus-resistant cucurbits have been engineered by silencing the potyviral helper component protease (HC-Pro) or even plant susceptibility genes such as eukaryotic translation initiation factors. For example, a combined HIGS strategy in melon targeted the eIF4E gene involved in cucumber vein yellowing virus (CVYV) infection; the transformed melon showed high resistance to CVYV. These examples demonstrate that coat proteins and related viral genes are effective HIGS targets.
3. Cucurbit HIGS has also been explored for fungi. Although stable transformation protocols are less routine for cucurbits, some advances have been reported. One group transiently silenced powdery mildew fungus genes in cucumber by agro-infiltration of dsRNA constructs, reducing fungal growth. Another promising angle is using root-expressed dsRNA to protect against soil-borne pathogens. For example, nicotiana plants engineered with cucumber sequence constructs were able to produce mobile small RNAs that limited a watermelon pathogen. These early trials suggest that as

genetic engineering tools improve for cucumber and squash, HIGS can be applied against oomycetes like *Phytophthora melonis* and fungi like *Podosphaera xanthii*. At present the cucurbit literature is dominated by antiviral examples, but the underlying principle is general: target a viral or fungal gene essential for infection, have the plant produce the corresponding dsRNA, and the pathogen's replication or virulence is suppressed.

4. Insects of cucurbits might also be targeted by HIGS. The green peach aphid (*Myzus persicae*), which vectors cucurbit viruses, could potentially be controlled by the same dsRNA lines that are effective in potato (e.g. dsMIF1), though no published study in cucumber exists yet. Whitefly (*Bemisia* spp.) RNAi constructs successful in tobacco or tomato [see previous section] could conceivably be expressed in melon or zucchini to interfere with whitefly survival. Although we lack published cases specifically in cucurbits, these approaches are likely “real or proposed” candidates. If used, the target genes would be similar to the Solanaceae examples (e.g. insect gut enzymes or virus RNAi suppressors).
5. In summary, HIGS in Cucurbitaceae is currently most advanced for viral disease control. Transgenic cucurbits targeting virus coat proteins or replication factors show complete or partial immunity. Efforts for fungi and insects are more nascent but follow the same concept: choose a critical pathogen gene (virulence factor, structural protein, replicase) and express its antisense. When evaluated, these are expected to reduce disease or pest spread.

HIGS in Brassicaceae Vegetable Crops

1. Brassicaceae (mustard family) includes cabbages, broccoli, kale, radishes, and turnips. These vegetables face their own set of challenges: cabbage aphids, clubroot (caused by *Plasmodiophora brassicae*), white rust, downy mildew, and fungal pathogens like *Sclerotinia sclerotiorum*. Since many crucifers are genetically related, approaches discovered in one species can often be transferred to others.
2. One exciting new development is the use of HIGS against clubroot. Clubroot is a serious soil-borne disease in crucifers, and no fully resistant cultivars exist. A recent 2025 study achieved proof-of-concept by transient HIGS in *Brassica rapa* (a close relative of cabbage). The researchers identified two *P. brassicae* effector genes, Pb48 and Pb52, that help the pathogen infect roots. They introduced hairpin constructs into susceptible brassica plants to silence these effector genes. The result was a clear

reduction in clubroot symptoms: silencing *Pb48* or *Pb52* individually significantly decreased the number of root zoosporangia (infection structures) and produced smaller root galls. Plants expressing either dsRNA showed downregulation of host hormone genes that facilitate gall formation, demonstrating disrupted disease development. This transient HIGS experiment validates that targeting pathogen effectors can thwart *P. brassicae*. A logical next step is to create stable transgenic cabbages or rapeseed expressing these dsRNAs, with the aim of durable clubroot resistance. The observed effects (smaller galls, less infection) underscore the practical potential of HIGS in brassicas.

3. Fungal pathogens are also targets. *Sclerotinia sclerotiorum*, the causative agent of stem rot and white mold in many crops, infects brassicas. Research (mostly in tobacco/Arabidopsis) suggests key *S. sclerotiorum* genes like oxaloacetate hydrolase (*SsOah1*) and MAP kinase regulators (*SsCak1*) can be silenced by HIGS to reduce virulence. By analogy, Brassica hosts engineered to express dsRNA against *SsOah1* or *SsCak1* should show increased resistance to sclerotinia. Indeed, transgenic Arabidopsis and tobacco targeting *SsOah1* exhibited significantly less disease. It is reasonable to expect that similar constructs in crucifer crops will confer some protection against white rot, an anticipated effect in our table. The key lesson is that HIGS against fungus virulence factors has worked in plant models, and brassicas can likewise silence those genes through transgenesis.
4. Brassicaceous vegetables are also plagued by insect pests like cabbage whitefly and aphids. Although no published case specifically in Brassica using HIGS is yet available, the principle remains. For example, if a cabbage cultivar were engineered with dsRNA against a whitefly gut or salivary protein gene (analogous to the tobacco/Bemisia case), the plant would likely suffer less feeding damage and virus transmission. Similarly, aphid-resistant *B. oleracea* lines expressing aphid target RNAs could reduce aphid populations. These ideas fall under proposed rather than realized applications, but they parallel successes in Solanaceae.
5. To summarize, HIGS in brassicas offers promising new defenses. The clubroot example shows how silencing pathogen effectors directly blocks disease. HIGS targeting fungal genes holds promise for white mold resistance. Insects and viruses could similarly be managed by appropriate dsRNAs. These cases support the idea that HIGS can be extended to any vegetable family by tailoring the gene targets.

HIGS in Fabaceae Vegetable Crops

- The Fabaceae (legume) family includes peas, beans, lentils, and also soybean (though soybean is often a grain legume, it shares pests with edible beans). Legumes face aphids, beetles, nematodes, and many viruses. HIGS has begun to make inroads here too, mostly focusing on viral and aphid pests.
- Viruses cause major losses in beans and peas. For instance, Bean golden mosaic virus (BGMV) devastates common bean in tropical regions. A recent HIGS example used transgenic bean plants expressing dsRNA targeting the virus replication gene (AC1). These plants showed strong resistance: viral DNA levels were dramatically reduced, preventing systemic spread. Other plant viruses in Fabaceae have been targeted similarly. For example, soybean mosaic virus (SMV) is a major soybean pathogen. Soybean plants transformed with hairpins against the SMV coat protein or HC-Pro genes exhibited near-complete resistance. Likewise, Cowpea severe mosaic virus (CPSMV) in cowpea (and even maize streak strains) has been prevented by HIGS of its coat protein gene in cowpea. These cases illustrate that transgenic legumes can be made virus-proof by silencing essential viral genes.
- Aphids are another Fabaceae pest group. The green peach aphid *M. persicae* also infests beans and peas. In potato (a Solanaceae but close enough to mention), HIGS silencing of aphid genes achieved high mortality, and a related study showed dsRNA against aphid cuticle proteins impaired reproduction. By extension, we can anticipate that transgenic peas or beans expressing such dsRNAs would reduce aphid damage. Although soybean itself is a grain crop, the findings from soybean studies are relevant; soybean plants expressing aphid-targeting RNAs saw reduced virus transmission by aphids, which is analogous to protecting edible beans.
- Other pests in Fabaceae include nematodes like root-knot nematode (*Meloidogyne*) and stink bugs, but specific HIGS examples are still emerging. Given the success against a range of viruses and aphids, it is likely that nematode and beetle control via legume-expressed dsRNAs will follow. For example, if soybean roots expressed dsRNA against a nematode effector, nematode infection should drop, a prediction supported by preliminary nematode RNAi studies in *Arabidopsis* and tomato.

Regulatory and Public Acceptance Considerations

1. Despite the technical successes, HIGS crops confront regulatory and public perception challenges akin to other genetically modified (GM) plants. Because HIGS typically involves inserting a new genetic construct into the host, it falls under GMO regulations in most countries. This means transgenic HIGS vegetables would require extensive safety testing and regulatory approval before commercialization. Many countries have strict controls: field trials of novel HIGS plants may be restricted, and consumer products might need labeling or face moratoria on cultivation.
2. Critics sometimes raise biosafety questions. They ask whether plant-produced dsRNA or siRNA could inadvertently silence non-target organisms (like beneficial insects or soil microbes). In practice, HIGS constructs are chosen for their sequence specificity; target genes are unique to the pest, and risk of off-target effects is low. Moreover, dsRNAs are naturally degraded in the environment. Scientific assessments generally find HIGS to be as safe as conventional GM insecticidal traits, if not safer, due to its high target specificity. Nonetheless, the involvement of transgenic technology triggers ethical and regulatory scrutiny. As one review notes, “HIGS faces biosafety concerns and ethical issues because it involves genetic modification... it is subject to regulatory and policy restrictions”.
3. Public acceptance is another factor. Surveys indicate that attitudes toward “biotech” foods vary widely. Some consumers may view HIGS crops with skepticism simply because they are GM. In contrast, other stakeholders appreciate the potential to reduce chemical pesticide use. Importantly, HIGS can sometimes be managed as part of integrated management: for example, the dsRNAs in plant tissue do not persist in the food or environment like chemicals might, which could be framed as an advantage.
4. Regulators are starting to address RNAi-derived products. In 2017, the U.S. EPA became one of the first authorities to approve a sprayable dsRNA product (for western corn rootworm on corn), recognizing dsRNA as a pesticide active ingredient. This set a precedent that dsRNA-based strategies can be regulated differently from GM crops. In 2020, the EPA approved a genetically modified soybean that produces dsRNA targeting an insect (whitefly). These steps suggest a possible path for HIGS: either as a GM crop regulated as a plant-incorporated protectant or, in some cases, as a non-GM dsRNA spray. For HIGS vegetables, if regulators classify them as conventional GMOs, the process will be lengthy and costly. If instead they accept analogies to RNA pesticides, HIGS traits might face fewer hurdles.

5. Overall, the regulatory and social landscape for HIGS is evolving. On one hand, strict GMO laws could slow adoption of HIGS in vegetables. On the other hand, the alignment of HIGS with sustainable agriculture (reduced pesticides, targeted action) may win public support over time, especially if communicated clearly. Importantly, because HIGS constructs produce no foreign proteins and only plant-like small RNAs, many scientists argue their risk profile is minimal. Continued dialogue with regulators and the public will be essential. Labeling, benefits to human health and the environment, and alternatives (like spray-induced RNAi) will factor into acceptance.

Conclusion

Host-Induced Gene Silencing represents a versatile and promising new tool for protecting vegetable crops. By repurposing the plant's RNAi system, HIGS can neutralize a wide array of threats – from insects and aphids to nematodes, fungi, oomycetes, and viruses – in a highly specific manner. The reviewed cases show that silencing just one or a few essential pest genes can dramatically reduce disease or damage. Transgenic tomatoes and potatoes have been shown to resist their most serious diseases when expressing the right dsRNA, and melon, squash, beans and cabbages have similarly benefited from targeted gene silencing of their foes. It is important to note that HIGS is not a panacea but rather a complement to other strategies. Effective use of HIGS requires choosing targets carefully (to avoid pest resistance), stacking multiple targets if possible, and integrating with good agronomic practices. Breeding can still be used in parallel, and HIGS crops should be part of holistic pest management. However, when used appropriately, HIGS can significantly lower the need for chemical pesticides and can tackle pests for which breeding has failed to find strong resistance. Regulatory and public acceptance hurdles remain. As with any GMO technology, HIGS crops must pass safety evaluations. Stakeholders must be convinced of their benefits and minimal risks. Encouragingly, HIGS constructs do not produce novel proteins in food (only small RNAs similar to those naturally present in plants) and thus pose no obvious human health hazard.

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