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Advances in Using Spray-Induced Gene Silencing (SIGS) for Managing Fusarium Head Blight in Wheat

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Abstract. Fusarium head blight (FHB), caused by Fusarium graminearum, severely impacts wheat yield and grain quality, while producing the mycotoxin deoxynivalenol (DON) that endangers human and animal health. Chemical control faces rising fungicide resistance, and breeding resistant varieties is time-consuming and challenged by rapid pathogen evolution. Spray-induced gene silencing (SIGS), a non-transgenic RNAi approach, offers a promising green alternative by applying exogenous dsRNA to silence key pathogenicity genes. This review summarizes recent SIGS progress for FHB management, covering its mechanism, target gene selection, validated targets, and field efficacy. And we also discuss efforts to optimize dsRNA delivery systems, with an emphasis on nanotechnology-based approaches aimed at prolonging persistence and enhancing targeting efficiency. Current studies show SIGS effectively reduces pathogen virulence and DON accumulation. Howeveer, field application is hindered by dsRNA instability, resistance risks, and inadequate environmental safety data. Future research may focus on multi-target resistance management strategies and the development of smart, stimulus-responsive nanocarriers to accelerate the translation of SIGS into sustainable FHB control programs.

Keywords: Fusarium head blight, Fusarium graminearum, spray-induced gene silencing, double-stranded RNA, nanodelivery systems

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important staple crops worldwide, providing a fundamental source of calories and protein for billions of people [1]. However, Fusarium head blight (FHB), caused by the fungal pathogen *Fusarium graminearum*, has long posed a serious threat to wheat production. During epidemic years, FHB can cause yield losses ranging from 10–30%, and in severe cases, complete crop failure [2]. More concerning is that *F. graminearum* produces mycotoxins such as deoxynivalenol (DON) and zearalenone (ZEN), which can enter the food chain and pose significant health risks to humans and livestock [3]. Today, chemical control measures have achieved some success in managing FHB on a global scale [4]. But with the continuous evolution of fungicide resistance and environmental contamination caused by pesticide residues, conventional chemical control is facing unprecedented challenges [4].

With the purpose of handle the problems, spray-induced gene silencing (SIGS), an RNA interference (RNAi)-based technology, has turned out to be a novel and environmentally friendly

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disease management strategy. SIGS involves the exogenous application of double-stranded RNA (dsRNA) onto plant surfaces, which is then taken up by the pathogen, triggering the RNAi machinery and it will lead to specific silencing of target gene expression, and then inhibiting pathogen growth and virulence. Different from host-induced gene silencing (HIGS), which relies on genetically modified crops, SIGS does not require transgenic manipulation, instead, it circumvents the ecological concerns associated with GMOs and offers greater flexibility and public acceptability. In recent years, researchers have made significant progress in understanding the mechanism of SIGS, selecting appropriate target genes, and evaluating its performance under field conditions. This review will systematically summarize these research advances, discusses current technical bottlenecks, and provides perspectives on future directions for the development of SIGS-based FHB management.

2. Mechanism of action of SIGS technology

SIGS is a novel, non-transgenic disease management strategy based on the principles of RNAi. The core mechanism of SIGS is as follows: exogenous dsRNA enters target cells and then the dsRNA is recognized and cleaved by Dicer endonucleases, generating siRNAs of 21–25 nucleotides in length. These siRNAs are then come together with Argonaute and other proteins into the RNA-induced silencing complex (RISC), which specifically recognizes and degrades homologous mRNA through complementary base pairing, thereby silencing target gene expression [5, 6]. In contrast to HIGS and virus-induced gene silencing (VIGS), SIGS achieves specific silencing of key pathogen genes simply by directly spray application of dsRNA, with no need for genetic modification of the crop. The typical SIGS workflow includes target gene selection and validation, design and large-scale synthesis of dsRNA, and field spray application. In 2016, Koch and his colleagues first demonstrated the feasibility of this approach by exogenously applying dsRNA targeting *Fusarium graminearum* and effectively suppressing disease in barley [7].

3. Target gene selection of SIGS technology

Choosing the right target genes is the first and most important step for successfully applying SIGS technology. An ideal target gene needs to have these benefits that meeting a few basic requirements. First, the gene should play an important role in pathogen survival, growth, pathogenicity, or mycotoxin production. Second, the gene sequence should be conserved among target pathogen populations but lack homologous sequences in non-target organisms, ensuring species specificity and minimizing ecological risks. Also, the gene should be expressed at a moderate level—not too high, not too low—so that the RNAi machinery can silence it effectively.

In the past few years, several research teams have conducted SIGS-based validation studies targeting key genes in *F. graminearum*. For example, spray application of a cocktail of siRNAs targeting five critical pathogenicity-related genes in *F. graminearum*—the protein kinase gene Gibberella pathogenicity mitogen-activated protein kinase 1 (Gpmk1), the zinc finger protein gene *Fusarium graminearum* CHY-type zinc finger protein 1 (FgChy1), the transcription factor F.g Sterol Regulator transcription factor (FgSR), the DON biosynthesis gene Trichodiene synthase gene(TRI5), and the cell end marker protein gene *Fusarium graminearum* cell-end marker protein TeaA (FgTeaA)—resulted in significantly reduced target gene expression and markedly inhibited pathogen spread in wheat leaves [6, 8]. Among these, the combination of all five siRNAs exhibited remarkable disease resistance effect.

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In the ergosterol biosynthesis pathway, the Cytochrome P450 Sterol 14α-Demethylase(CYP51) family genes represent important candidate targets. The *F. graminearum* genome contains three paralogous genes encoding sterol 14-α demethylase—CYP51A, CYP51B, and CYP51C. Studies have shown that dsRNA targeting CYP51 genes effectively reduces target gene expression and inhibits fungal growth in vitro. Höfle and colleagues systematically compared the silencing efficiency of dsRNAs of different lengths targeting CYP51 genes. Their research provides important references for dsRNA design [9].

More recently, global regulatory factors that control fungal secondary metabolism and development have also got attention. Stakheev et al. targeted *Fusarium graminearum* velvet protein 1(FgVe1), a gene regulating fungal growth, mycotoxin production, and pathogenicity [10]. They found that dsRNA treatment result in a 19.78-fold silencing of FgVe1 expression, accompanied with suppressed expression of genes involved in trichothecene and aurofusarin biosynthesis. At last, it results in reduced DON accumulation and altered culture pigmentation [10].

In addition to directly aiming at pathogen genes, SIGS can also enhance disease resistance with the method such as silencing negative defense regulators in the wheat. For example, silencing genes such as 9-lipoxygenase (9-LOX) and TaSSI2 has shown to enhance wheat's antioxidant capacity and resistance to pathogen attack. This "dual-targeting" strategy—simultaneously silencing pathogen virulence genes and host negative defense regulators—gives a new avenue for improving the overall efficacy of SIGS-based disease control.

4. The field control effect of SIGS on wheat scab

As an emerging green disease management strategy, SIGS technology has demonstrated remarkable efficacy in controlling various crop diseases. In experiments by Ren and colleagues, spray application of dsRNA targeting ten specific genes in rapeseed led to a reduction of leaf lesion area by over 50% [5]. Furthermore, SIGS has also shown outstanding performance against the difficult-to-control gray mold disease [11]. Research on the application of SIGS for managing Fusarium head blight (FHB) in wheat has gradually progressed from laboratory settings to greenhouse and field environments. Collectively, these findings indicate that SIGS holds great promise for the control of FHB in wheat.

In 2025, researchers tested SIGS technology in wheat fields to fight the fungus, *Fusarium graminearum*. They picked three target genes, inserted them into plasmids, and used a special E. coli strain to produce dsRNA. After confirming in the lab that the fungus and wheat leaves could take up the dsRNA, they sprayed dsCHS3b or dsMGV1 in the field. Both worked very well—each one alone lowered the fungus population to below 0.1, which was much more effective than the fungicide tebuconazole. In addition, DON toxin levels in harvested grain were reduced by 90%, indicating excellent control efficacy of the dsRNA treatment [12]. This study provides critical proof-of-concept for using SIGS to combat FHB in wheat and represents a milestone achievement in the field. However, there should be more studies about the field Efficacy, not one, not two, not even ten. After that, we can believe in SIGS used in FHB more firmly

5. Nanodelivery

Although SIGS technology has demonstrated huge antifungal efficacy under laboratory conditions, the instability of dsRNA in field environments remains a major barrier to its large-scale application. Naked dsRNA is susceptible to ultraviolet (UV) degradation, rain wash-off, and microbial nuclease degradation. These problems result a short persistence period and inconsistent disease control

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performance [13]. Furthermore, according to several studies, the success of SIGS in plant disease management is highly dependent on the efficiency of pathogen RNA uptake [14]. In the application of SIGS, the efficacy of fungal infection inhibition is negatively correlated with dsRNA length [9]. In order to enhance the disease control efficacy of dsRNA and broaden its activity spectrum, it is often necessary to incorporate multiple dsRNA fragments, which inevitably increases dsRNA length and consequently reduces pathogen uptake efficiency.

Nanodelivery systems offer an innovative strategy to address this bottleneck: nanocarriers not only protect dsRNA from environmental degradation but also enhance dsRNA bioavailability by improving foliar retention, promoting cellular uptake, and enabling controlled release [13]. There were current nanocarriers used for SIGS primarily include polymer nanoparticles, mesoporous silica nanoparticles (MSNs), inorganic nanoparticles such as layered double hydroxides (LDH), and biologically derived carriers [15]. Findings suggest that combining calcium phosphate (CaP) with dsRNA to form CaP:dsRNA complexes extended the effective duration of control compared to naked dsRNA in ex vivo leaf assays. After 7 days, while the efficacy of naked dsRNA treatments began to decline, the nanocomplexes continued to exhibit effective silencing. However, naked dsRNA performed better at early time, because of more efficient uptake, maybe [10].

On the one hand, some carriers may reduce dsRNA uptake efficiency by the pathogen, but on the other handnanocarrier association clearly extends the effective persistence of dsRNA, and certain carriers can even promote dsRNA uptake efficiency. Overall, by leveraging nanodelivery technology, SIGS shows a promised future for substantial improvements in both the duration of efficacy and disease control performance.

6. Conclusion

As a non-transgenic RNAi-based strategy, SIGS technology offers unique advantages and substantial application potential for the green control of Fusarium head blight (FHB) in wheat. Over the last decade, significant progress has been made in target gene selection, dsRNA design optimization, and nanodelivery systems, with multiple research teams successfully demonstrating the feasibility of SIGS for FHB management in wheat. What's more, the transition of this technology from laboratory settings to large-scale field application still faces several challenges, including dsRNA environmental stability, resistance risk, ecological safety, and cost-effectiveness. The deep integration of nanotechnology with SIGS provides an innovative pathway to overcome these bottlenecks, with smart delivery systems expected to significantly improve the stability and persistence of field disease control. Looking forward, with continued advances in target gene discovery, delivery technology innovation, and reduced production costs, SIGS is suggested to be an indispensable green tool within integrated FHB management programs. And it will make important contributions to ensuring food security and promoting sustainable agricultural development.

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