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Synthetic Gene Drives in Plants: Development Strategies, Potential Applications, and Ethical Considerations

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Received: 23 April 2024 | **Revised:** 16 June 2024 | **Accepted:** 25 July 2024

Funding: This work was supported by grants from Project for Young Scientists in Basic Research (YSBR-078, to W.Q.) from the Chinese Academy of Sciences, the National Key R&D Programme of China (2019YFA0903900, to W.Q.), and the Strategic Priority Research Programme (Precision Seed Design and Breeding, XDA24020103, to W.Q.).

ABSTRACT

We are facing significant agricultural and environmental challenges, including herbicide-resistant weeds that compromise crop yields and invasive plant species that disrupt local ecosystems. Traditional crop breeding methods are increasingly seen as ineffective in the face of these issues. One innovative approach involves the use of gene drives, a technology that enables targeted genetic modifications to transmit at a super Mendelian rate (>50%) and therefore, rapidly propagate through natural populations. In this perspective, we introduce the principles underlying our development of a plant synthetic toxin-antidote drive, *CAIN* (CRISPR-Assisted Inheritance utilising *NPG1*). We evaluate the potential of gene drives to alter the genetics of weed populations or suppress invasive plant populations. We explore various factors that influence the effectiveness of gene drives, including the emergence of suppressor mutations and life-history traits such as sexual and asexual reproduction patterns, seed and pollen dispersal methods, and the variability in plant generation times. Additionally, we discuss the biosafety concerns related to gene drive experiments and field releases, strategies to counteract unwanted gene drives, and the ethical implications, particularly considering the risk of the intentional misuse of gene drive technology and the need for robust regulatory and monitoring frameworks.

Now a days we face a complex array of challenges that threaten both food security and environmental sustainability. One significant issue is the ongoing battle against agricultural weeds, particularly those that have developed resistance to commonly used herbicides. The proliferation of herbicide-resistant weeds often leads to increased herbicide use, which can harm biodiversity and diminish agricultural productivity. For example, herbicide resistance in the weed *Alopecurus myosuroides* (blackgrass) costs England £0.4 billion annually, and globally, with more than 200 known herbicide-resistant weeds, the cost could be significantly higher [1]. Another issue is the environmental concerns posed by invasive plant species. These plants, often introduced into new environments without their natural predators, can spread uncontrollably, disrupt local ecosystems, and lead to the decline of native plant and animal species. Globally, the economic cost of invasive species over the past 50 years was estimated to be $1.3 trillion in 2021 [2]. Together, these challenges demand urgent and innovative strategies to safeguard our agricultural productivity and preserve environmental balance.

The development of genetic manipulation tools could offer strategies for addressing some of these toughest challenges in agriculture and ecology. Given that most herbicide targets are well understood and numerous mutations conferring resistance

Summary

- Synthetic toxin-antidote gene drives can achieve over 50% transmission efficiency in plants, offering a new tool for agricultural weeds and invasive species control.
- The development of synthetic gene drives must take into account the potential for suppressor mutations and the distinct life-history traits of the target plant species.
- Biosafety and ecological considerations are crucial for the responsible deployment of synthetic gene drives.

have been identified, it is technically feasible to restore herbicide sensitivity in resistant weed populations [3]. Additionally, by disrupting genes crucial for fertility, scientists can potentially reduce weeds or invasive populations. However, integrating traits that are advantageous to humans into wild plant populations faces significant hurdles due to the principles of Darwinian selection. Traits that benefit humans, such as reduced fertility in weeds, are likely detrimental to the plants themselves, causing these traits to disappear from the population over time as they confer a survival disadvantage. This process is fundamentally different from traditional agriculture, where new plant varieties are selectively bred and maintained under controlled conditions to ensure that they thrive.

The existence of selfish genetic elements, which can pass on their genetic material to offspring at rates exceeding the typical 50% expected by Mendelian inheritance in heterozygotes, suggests that even harmful mutations could spread throughout a population if they can circumvent Mendel's genetic rules [4–6]. Such elements include genes encoding homing endonuclease, which creates a double-strand break (DSB) at the homologous location on the homologous chromosome. This DSB is then repaired by homology-directed repair (HDR), effectively converting heterozygous germline cells to homozygous ones through a 'copy-and-paste' mechanism—a process known as homing (Figure 1A) [7]. This mechanism guarantees that homing endonuclease genes can propagate through successive generations in an outcrossing population. The ability of such elements to propagate in a population despite potential disadvantages to the host organism suggests a novel approach for managing genetic materials of wild plant populations [8, 9].

Drawing on these natural phenomena, synthetic gene drives are being proposed and developed to spread genetic modifications across natural populations [6, 10–12]. Currently, the most commonly proposed synthetic gene drive systems are those that utilise CRISPR technology to mimic the natural process performed by homing endonucleases (Figure 1B). While homing endonucleases are difficult to adapt for use in new species or at new genetic locations, CRISPR-Cas9 overcomes these limitations by enabling precise DNA cleavage at specific genomic positions directed by guide RNAs (gRNAs). This design has successfully facilitated rapid gene spread in various organisms, including fungi, mosquitoes, flies, and mice [12]. However, homing-based gene drives often lead to the creation of resistance alleles [13, 14], which arise through non-homologous end joining (NHEJ) DNA repair pathway. This pathway functions without the 'copy-and-paste' mechanism and results in small indels that are resistant to further DNA cleavage, thereby preventing gene drives from spreading further. Since plants predominantly use NHEJ repair pathways [15], this poses a significant challenge for the effective and sustained application of homing-based gene drives in plant populations.

To develop an effective synthetic gene drive for plants, one might propose to bypass reliance on the HDR pathway altogether. This can be achieved using toxin-antidote gene drives, which are inspired by naturally occurring systems found in a broad range of species [16] including plants [17, 18]. In these systems, a toxin gene is expressed before meiosis and affects all gametes, disrupting normal gamete development. The antidote, which is genetically linked to the toxin, is activated after meiosis, neutralising the toxin's effects and ensuring that only the gametes or zygotes carrying the toxin-antidote drive survive (Figure 1C). This selective survival gives a significant inheritance advantage to the toxin-antidote system, making it a promising and transformative strategy for gene drive development in plants.

Natural toxin-antidote systems rely on a sophisticated balance of stoichiometry and the timing of expression between the toxin and the antidote, which may be difficult to transfer across various species. Nevertheless, CRISPR-Cas9 can be used to create a universal template that mimics the toxin-antidote strategy across species [19–21]. Specifically, the CRISPR-Cas9 system acts as the toxin by targeting and cleaving an essential gene, thereby creating loss-of-function alleles that disrupt normal biological processes. Simultaneously, a modified version of the same essential gene, engineered to be resistant to Cas9, serves as the antidote compensating for the loss of function caused by the CRISPR-induced knockout (Figure 1D). As a result, only the cells that inherit the drive can survive and function properly, promoting the spread of these synthetic elements (and any genetically linked 'cargo') through the population.

Synthetic toxin-antidote gene drives can largely be divided into two major categories based on the developmental stages and cell types they target (Figure 2A). Toxin-Antidote Recessive Embryo (TARE) drives [20, 21], also known as Cleave and Rescue (*ClvR*) [19], target haplosufficient genes essential for zygotic development, causing the death of offspring that inherit two loss-of-function alleles but lack the gene drive, ensuring only progeny with the drive allele survive. Conversely, Toxin-Antidote Dominant Sperm (TADS) drives specifically target genes that are critical for spermatogenesis and expressed during the haploid stage [21]. A plant with a TADS drive pollinating a wild-type plant results in only TADS-carrying pollen grains completing fertilisation, achieving 100% transmission of the drive. TADS is potentially more effective than TARE drives as eliminating half of the haploid pollen grains may not significantly impact fertility, unlike the substantial fertility reduction seen with the loss of ovules or embryos (Figure 2B), thus enhancing the spread of the gene drive in the population (Figure 2C).

In our recent study, we developed a synthetic toxin-antidote gene drive for *Arabidopsis thaliana*, which we named *CAIN* (CRISPR-Assisted Inheritance utilising *NPG1*). The core

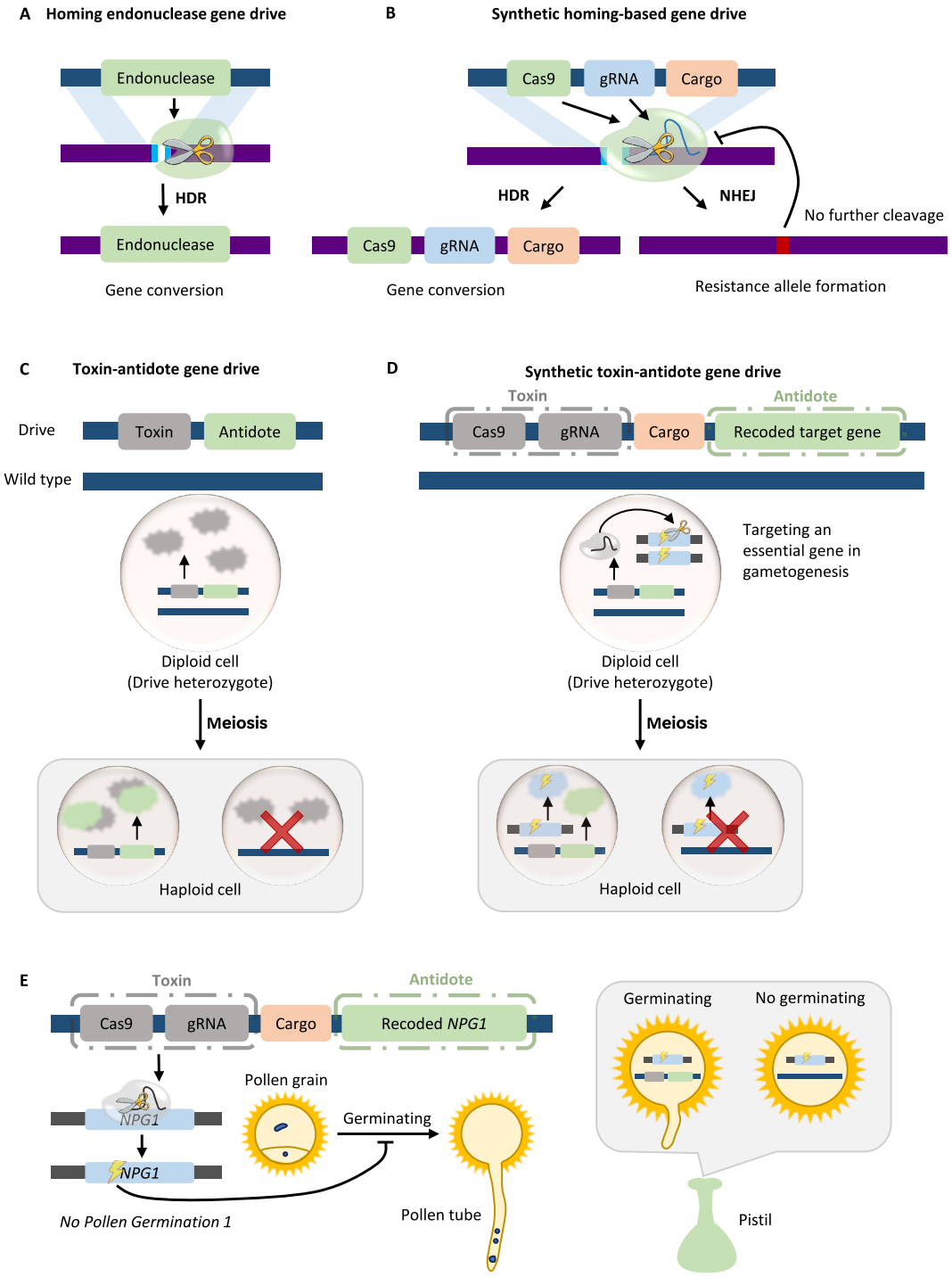

FIGURE 1 | Design principles for synthetic gene drives. (A) An endonuclease cleaves the wild-type allele, inducing a double-strand break (DSB) that is repaired via homology-directed repair (HDR), leading to gene conversion. (B) A Cas9-gRNA complex cleaves DNA at specific sites, imitating a natural homing endonuclease mechanism. However, DSBs repaired through non-homologous end joining (NHEJ) will generate resistance alleles that prevent further Cas9 cleavage. (C) A toxin gene expressed pre-meiosis impacts all gametes, disrupting their development. The genetically linked antidote is activated post-meiosis to reverse the toxin's effect. (D) Synthetic toxin-antidote drive utilises a gRNA-Cas9 complex to disrupt an essential gene (acting as the toxin) and employs a recoded, CRISPR-resistant version of this gene as the antidote, effective only in gametes harbouring the gene drive. (E) The design of the *CAIN* gene drive system. Pollen grains with only the toxin fail to germinate due to the disruption of *NPG1* (*No Pollen Germination 1*), while those with the antidote (recoded, CRISPR-resistant version of *NPG1*) progress to germinate normally, highlighting transmission bias through pollen development.

mechanism involves a gRNA-Cas9 cassette that targets and cleaves the *No Pollen Germination 1* (*NPG1*) gene, effectively acting as the toxin by preventing pollen germination (i.e., TADS type). A recoded, Cas9-resistant version of the *NPG1* gene serves as the antidote, functionality exclusively in pollen cells that carry the gene drive (Figure 1E). *CAIN* demonstrated

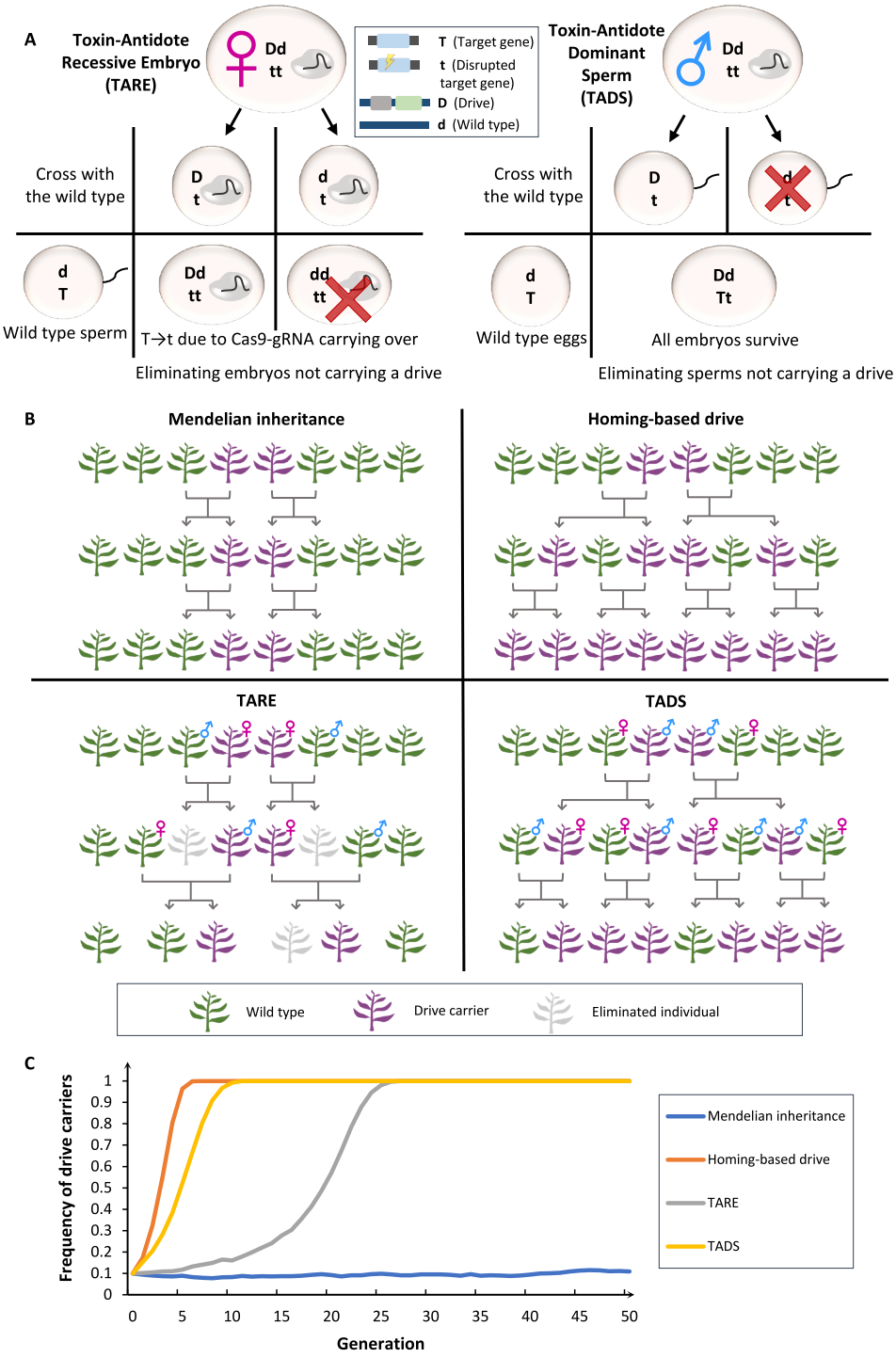

FIGURE 2 | Comparative analysis of gene drive mechanisms and outcomes. (A) Illustration of the differences in cellular mechanisms between toxin-antidote recessive embryo (TARE) and toxin-antidote dominant sperm (TADS) gene drives. (B) The propagation processes of homing-based, TARE, and TADS gene drives in a population. Note that the population size may remain constant due to environmental carrying capacity, suggesting that the number of individuals may not decrease with the implementation of TARE (as appeared in the figure), due to reduced competition for survival. (C) The dynamics of simulated populations for homing-based, TARE, and TADS gene drives over time. The simulation was performed using an individual-based, stochastic model derived from the Wright-Fisher process. This assumes a finite, randomly mating population reproducing in discrete, non-overlapping generations. Initially, the population comprised 90% wild-type individuals and 10% gene drive carriers (heterozygotes). For the homing-based drive, the drive allele converted wild-type alleles with 100% efficiency. The TARE gene drive exhibited a 100% cleavage efficiency in both female germline and embryos, and the TADS gene drive displayed a 100% male germline cleavage efficiency. In the TARE gene drive system, embryos that carry two copies of the disrupted target gene without the drive allele were aborted. Similarly, in the TADS gene drive system, male gametes carrying the disrupted target gene without the drive allele failed to fertilise.

remarkably high transmission rates, achieving between 88% and 99% over two successive generations, with minimal production of resistance alleles [22]. The mathematical modelling suggested that *CAIN*'s presence in a population could escalate from 1% to 99% in just about 17 outcrossed generations, demonstrating *CAIN*'s potential as a powerful tool for altering natural plant populations. *CAIN* stands out as the experimentally demonstrated gene drive of the TADS type and as the pioneering synthetic gene drive applied to plants.

The cargo gene carried by the gene drive system is versatile and can be tailored to address specific agricultural and ecological challenges, depending on the desired goals. Potential cargos for real-world application include genes that enhance a plant's sensitivity to herbicides. However, maintaining the evolutionary stability of these modifications in natural populations could be challenging due to their strong fitness cost upon the usage of herbicides. A better strategy is probably inserting the gene drive into a gene conferring herbicide resistance, thereby inactivating it. Through the spread of the drive, herbicide resistance is eradicated in natural weed populations (Figure 3A).

Theoretically, the gene drive system also has the potential to enhance the inheritance of beneficial traits, such as genes conferring drought or disease resistance [23, 24]. This could significantly bolster the resilience and survivability of

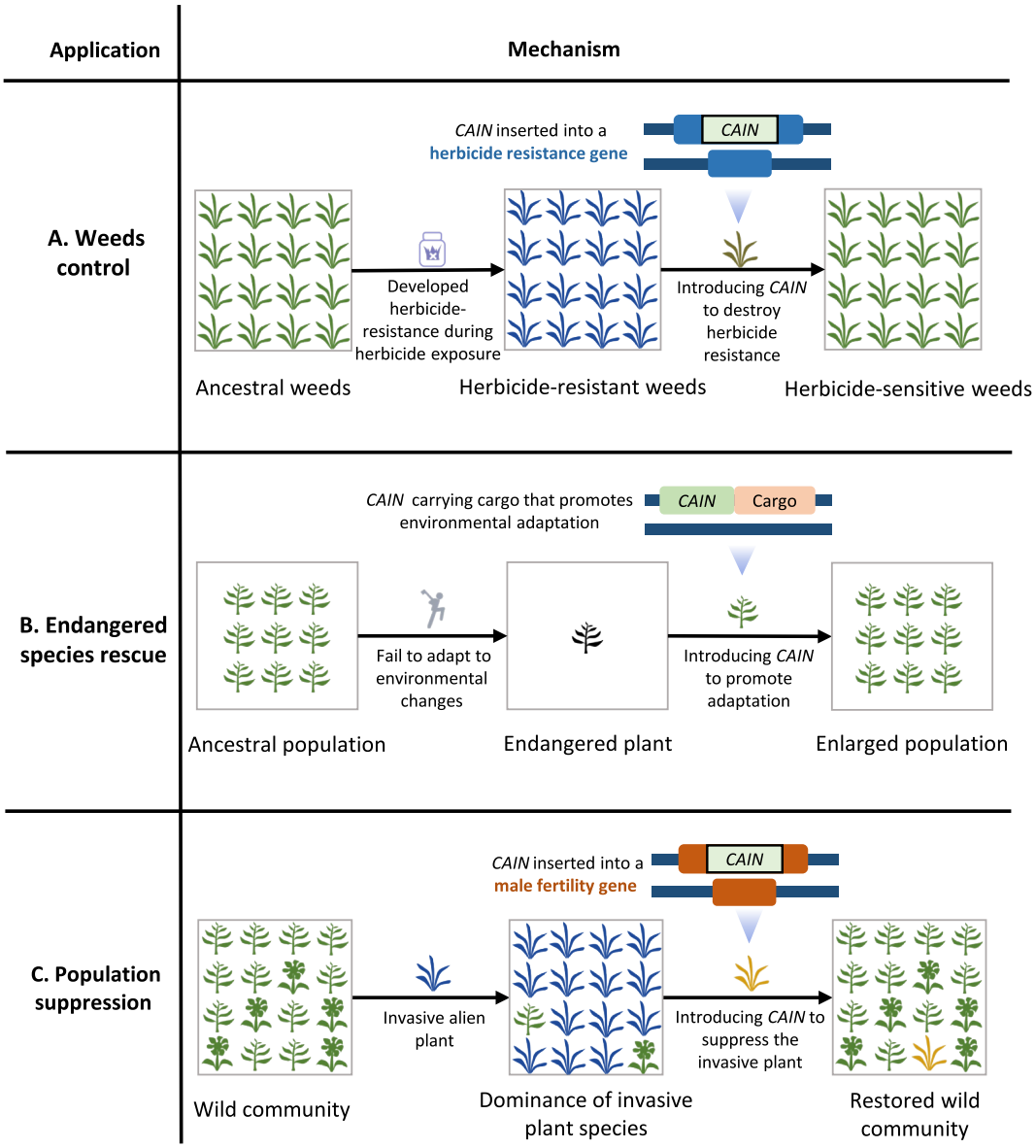

FIGURE 3 | Potential applications of the *CAIN* gene drive across different ecological and agricultural scenarios. (A) *CAIN* is specifically inserted into a herbicide resistance gene in herbicide resistance weeds to convert them back to herbicide-sensitive. (B) *CAIN* aids endangered plants by inserting genetic modifications that enhance their environmental adaptability. (C) *CAIN* suppresses populations of invasive alien plants by disrupting a haplosufficient male fertility gene that functions sporophytically, ultimately restoring the wild community. The mutated version of the targeted gene should be recessive.

endangered plant species in their natural habitats (Figure 3B). However, the advantage of gene drives typically appears when selection opposes the trait being driven. It is therefore crucial to evaluate whether the accelerated spread of such traits via the gene drive system outweighs the potential risks of introducing these genes into areas where they might not be needed. This strategy is likely more effective when introducing a gene that provides organisms with benefits for foreseeable challenges, thereby pre-adapting the population to stresses anticipated to arise in the near future.

Notably, *CAIN*'s faster propagation compared to other toxin-antidote drive designs, such as TARE-type drives (Figure 2C), underscores its potential for population suppression. This is achieved by targeting a haplosufficient male fertility gene, essential during the diploid stage of development (Figure 3C). This strategy leads to an initial surge in the number and frequency of heterozygous *CAIN* carriers, followed by population decline and potential extinction due to an increase in the male homozygous *CAIN* plants which were incapable of producing viable pollen grains. It is important to note that if the *CAIN* construct is inserted into a female fertility gene or viability gene instead of a male fertility gene, the population would not completely collapse due to the inability to fully eliminate the wild-type allele from the population [22].

Our proof-of-concept demonstration of *CAIN* in *A. thaliana* establishes a foundation for its application in other plant species. This possibility is supported by the fact that *NPG1*, a critical component of our system, is highly conserved across a wide range of monocots and dicots [25]. For example, its rice homologous gene, *OsPCBP*, is expressed in haploid pollen grains and plays a crucial role in pollen maturation [26], underscoring *CAIN*'s potential adaptability to other plant species.

Nevertheless, implementing gene drive systems in real-world scenarios faces additional challenges, including technical limitations, the emergence of suppressor mutations, and the diverse life history traits present among plant species. A significant challenge is establishing transformation protocols for various plant species; while some model plants already have established protocols, adapting these to new plant species is not always straightforward [27]. Another technical challenge is transgene silencing, which frequently occurs in many plant species [28]; this is problematic because silenced gene drives are unable to propagate further. Future research should therefore focus on developing tailored transformation strategies for various plant species and on minimising gene silencing to enhance the effectiveness of gene drives over multiple generations.

The practical implementation of *CAIN* gene drives in real-world scenarios also necessitates careful consideration of potential suppressor mutations, which are genetic alterations that can suppress the spread of a gene drive. A major concern for *CAIN* is the development of a mutation at the *NPG1* locus that produces a cleavage-resistant, yet functional, *NPG1* allele (Figure 4A).

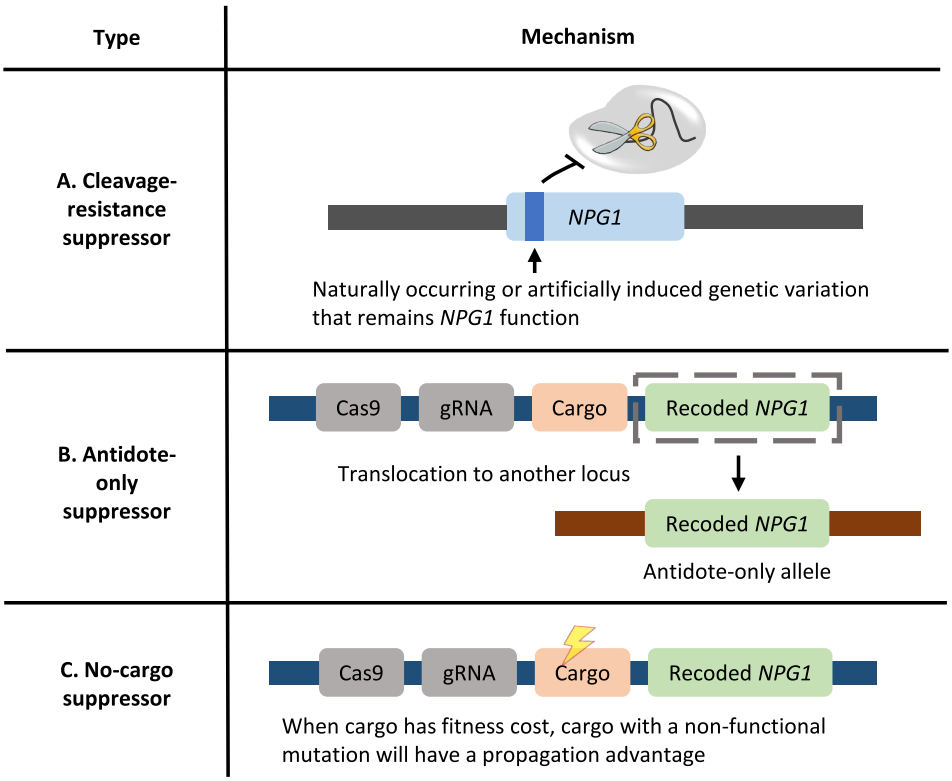

FIGURE 4 | Mechanisms of suppressor mutations of the *CAIN* gene drive. (A) A suppressor mechanism where a variant of *NPG1* gene is resistant to cleavage, maintaining its function despite the presence of a gene drive. (B) A suppressor mechanism where only the antidote allele is translocated without the associated cargo, forming an antidote-only allele. It can suppress a toxin-antidote gene drive by providing resistance against the toxin without the associated fitness costs of carrying the drive and the associated cargo. (C) A suppressor mechanism where the cargo has undergone a non-functional mutation. Such an allele can suppress the gene drive because it lacks the cargo, which often imposes a fitness cost.

Such an allele could hinder the spread of *CAIN*, as pollen carrying this allele would germinate even if it does not carry the *CAIN* drive. To minimise the likelihood of such resistance alleles forming, it is recommended for future applications to utilise multiple, highly efficient gRNAs to ensure frame-shifting mutations occur at least at one cleavage site. Additionally, targeting regions that are functionally important is advisable, as even point mutations that do not alter the reading frame could still possibly lead to non-functional *NPG1* alleles.

Other types of suppressor mutations could also impact the effectiveness of the *CAIN* system. For example, the recoded *NPG1* allele could rearrange itself out of the *CAIN* locus, resulting in a situation where only the antidote is present, thereby creating an antidote-only suppressor (Figure 4B). In applications aimed at modifying populations, loss-of-function mutations within the cargo gene—particularly those that carry a fitness cost—could undermine the gene drive by removing cargo-carrying alleles from the population (Figure 4C). While these suppressors might seem to diminish the effectiveness of the *CAIN* system, they also render the gene drive self-limiting, potentially confining its impact to temporary or localised changes in the genetic structure of wild populations. This inherent limitation could be advantageous in managing the ecological risks associated with gene drives.

When implementing a gene drive system in wild plant species, it's crucial to consider specific life-history traits that could influence its efficacy. For example, the efficacy of gene drive spread relies heavily on sexual reproduction; consequently, asexual reproduction, which occurs in many plant species, will diminish the spread efficiency of gene drives. Self-fertilisation in monoecious plants could also decelerate its spread because gene drive can bias inheritance only in heterozygotes while self-fertilisation increases the frequency of homozygotes. For the same reasons, self-incompatibility in plants, a biological mechanism that prevents inbreeding by inhibiting fertilisation between closely related individuals, will promote the spread of gene drives by encouraging cross-pollination and increasing heterozygosity. Additionally, seed and pollen dispersal methods—such as through wind, animals, or water—might influence dispersal distances and, consequently, the speed of gene drive spread. Collectively, all factors that increase the frequency of homozygotes limit the efficiency of gene drives.

Furthermore, reducing pollen count, especially in scenarios where stigmas receive pollen from multiple donors, could decrease the fertility of drive carriers and slow the gene drive's propagation. The range in plant generation times, from weeks to centuries, also affects the rate of gene drive spread (measured in absolute time rather than generations), with longer generation times leading to slower dispersal. Seed dormancy offers protection against gene drives, as a proportion of individuals are preserved in seed banks, which remain unexposed to gene drives for a relatively long period. These considerations highlight the importance of understanding and integrating the specific reproductive and ecological dynamics of target plant populations when planning to deploy gene drive systems.

Gene drives have the potential to spread to natural populations, which are shared by all humans, and may not always behave as originally designed and intended. This is especially relevant for plants due to the widespread occurrence of cross-species hybridisation [29], which increases the likelihood of gene drives being transmitted to different plant species. Consequently, there is an urgent need for the scientific community, policymakers, and stakeholders to engage in discussions about the responsible development and use of gene drive technologies [11, 30–32]. We will discuss three aspects related to the ethical considerations associated with the use of this technology, the design and experiments, the monitoring of clandestine synthetic gene drives, and the counteract strategies.

Prioritising biosafety in laboratory experimentation is essential. Indeed, in our proof-of-concept study we have chosen to demonstrate the efficacy of *CAIN* in *A. thaliana*, a self-pollinating model plant, to minimise the risk of unintended spread of the gene drive [22]. Further, the design of *CAIN* enables high specificity by targeting specific genotypes or ecotypes, which is achieved by selecting gRNA target sites that exploit naturally occurring genetic polymorphisms. This methodology allows for precise genetic modifications tailored to particular genetic backgrounds, ensuring that the gene drive affects only intended targets. Such precision provides the flexibility to apply the *CAIN* system in a controlled manner, which is vital for ensuring the safe and responsible deployment of gene drives.

While self-containment strategies are critical, they may not be sufficient in preventing the intentional misuse of gene drive technology, particularly when targeting wild plants and crops. This highlights the complexity of managing gene drives and emphasises the need for robust regulatory frameworks and continuous monitoring of the genetic makeup of wild populations to mitigate potential risks. Continuous oversight is particularly critical in countries where seeds are farmer-saved rather than commercially distributed, as these seeds may not undergo regular scrutiny. Furthermore, transparent investigation of gene drive mechanisms is crucial to understanding potential impacts and identifying molecular markers for detecting clandestine gene drives that could threaten food security.

To mitigate the risk of gene drive misuse, it is also essential to develop technologies that can counteract the spread of gene drive alleles. A promising strategy is the creation and potential release of suppressor lines, when necessary. For gene drive designs similar to *CAIN*, a straightforward and efficient method involves editing the native *NPG1* allele to make it resistant to Cas9 cleavage. This allele will increase in frequency after release into the natural population because it is resistant to gene drives and does not incur the fitness costs associated with the drive and linked cargo. Alternatively, engineering a new gene drive that targets the original cargo genes with the CRISPR/Cas9 system could also be useful. Therefore, the capability to quickly develop suppressor strains is a crucial component of a comprehensive risk management strategy for gene drives.

Forty-five years ago, James F. Crow remarked, 'Mendelian inheritance is a marvellous device for making evolution by natural selection an efficient process' [33]. Indeed, gene drives, by overriding Mendelian inheritance, can reduce the power of natural selection, thereby possibly spreading alleles deleterious to plants into natural populations. As we explore this emerging

area of gene drive, systems like *CAIN* have the potential to significantly alter ecological management and agricultural practices. The efficiency of gene drives remains a critical concern, particularly due to the potential for suppressor mutations and the complex life-history traits of different plant species. Concurrently, it is essential to address biosafety and ethical issues surrounding gene drive research and releases, as well as to develop methods for controlling the spatial and temporal spread of gene drives and techniques to reverse their propagation in populations, when necessary.

Author Contributions

Yawen Liu: visualization (equal). **Bingke Jiao:** formal analysis (equal). **Wenfeng Qian:** conceptualization (equal), writing–original draft (equal).

Acknowledgements

We thank Dr. Yang Liu for her constructive comments on the manuscript. This work was supported by grants from Project for Young Scientists in Basic Research (YSBR-078, to W.Q.) from the Chinese Academy of Sciences, the National Key R&D Program of China (2019YFA0903900, to W.Q.), and the Strategic Priority Research Program (Precision Seed Design and Breeding, XDA24020103, to W.Q.).

Conflicts of Interest

B.J. and W.Q. have been granted a China invention patent (ZL202311247476.0) based on some results discussed in this paper. The remaining authors declare no competing interests.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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