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Insect resistance management through Genome Editing and Gene Stacking method

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Abstract: Sustainable agricultural production is endangered by several ecological factors, such as drought, extreme temperatures, excessive salts, parasitic ailments, and insect pest infestation. As with other biotic stress factors, insect pests have also posed serious concerns related to yield losses due to which agricultural productivity is at stake. Several gene editing strategies are being executed with continuous emergence of variants. The CRISPR/Cas9 genome editing technology has shown great promise for quickly addressing emerging challenges in agriculture. It can be used to precisely modify genome sequence of any organism including plants to achieve the desired trait. Compared to other genome editing tools such as zinc finger nucleases (ZFNs) and transcriptional activator-like effector nucleases (TALENs), CRISPR/Cas9 is faster, cheaper, precise and highly efficient in editing genomes even at the multiplex level. Gene suppression via RNA interference (RNAi) provides an alternative strategy for insect pest management. The ingestion by insects of double-stranded RNAs targeting essential insect genes can trigger RNAi and lead to growth inhibition, developmental aberrations, reduced fecundity, and mortality. The insects are remarkably adaptable and can develop resistance to any control tactics, including transgenic plants containing multiple Bt toxins and RNAi. The innovations like genetically modified Bt toxins and discovery of insecticidal proteins from bacteria other than Bt will continue to provide new tools for pest control. The MGPS-based crops will be more durable with compliance of high refuges and other control tactics.

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Introduction

Climate change not only affects crop production through altered weather patterns, but also via increased environmental stresses such as soil salinity, drought and emergence of new disease and insect-pests. To keep up with the pace of population growth, it has recently been estimated that food production will need to be increased by 50% by 2030 and by 70–100% by 2050 for a well-fed world population (Zafar et al., 2020; Zafar et al., 2021; Zafar et al., 2021a). Pathogens, herbivores, and weeds cause ubiquitous problems for crop production, including 11%–59% losses in yields of the major crops in the world (Oerke, 2006). While resistance traits in wild plants are molded and remolded by natural selection, this evolutionary response has become skewed in agricultural systems since breeding for high yield and good quality has (consciously or unconsciously) removed such traits in crops (Zhan, Thrall, Papaïx, Xie, & Burdon, 2015). Most of the economically important crops suffer a wide range of yield losses despite operating control measures. Farmers have however adopted chemical pesticides for the control of insect pests, the widespread use of which can be deleterious to mankind and the environment. Reducing the burden of insect crop pests is a key priority, particularly since an estimated 70–100% increase in global food production will be required by 2050 to feed the burgeoning human population and that these changes in agricultural production will involve increased automation and biotechnological innovation (Zafar et al., 2020a). With the advent of genetic engineering and plant biotechnology, pest management has reached new horizons. Genetic modification of a range of crop plants with several insect resistance genes and continued implementation of Bt gene introgressed crops have shown trustworthy impact on productivity and sustainability of the technology. However, development of resistance in the insect pests to the Cry toxins has been a major apprehension. 2 Mutation(s) in the genes encoding for receptor molecules distracts interaction between the insect and the toxin, resulting in the emergence of the resistant insect-pest population. To deliver extended efficacy against insect pests and avert resistance, chimeric toxins developed by domain swapping of toxins and usage of combinatorial ICPs are also being exploited (Zafar et al., 2020a). This review addresses recent research advances that are underpinning strategies to enhance crop resistance to insect pests or that have the potential to do so.

Genome editing through CRISPR/Cas9 technology

Genome editing allow plant breeders to manipulate crop genomes at the nucleotide level with high precision. In particular, the advent of prokaryotic-derived Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR associated protein (Cas) systems and its use in plant genome editing has been a crucial turning point towards a new era of crop breeding. Cas9 and Cas12a, are two popular RNA guided engineered nucleases (RGENs) which mediate genome editing, directed by the sequence-specific pairing of a guide RNA (gRNA) to the target DNA (Jinek et al., 2012). The pace of discovery, both of essential insect traits and of reagents to perturb these traits, has increased dramatically through molecular biology and genomics, and opportunities for interventions are continuing to expand as technologies (e.g., sustained expression of multiple stacked transgenes in plants) are optimized and as new technologies (e.g., editing of plant genomes by CRISPR) are introduced. It is now routine to address crop resistance to insect pests in molecular terms. Specifically, the insect trait is defined in terms of one or multiple gene targets, and plant resistance is defined as a gene or suite of genes with a product or products that inactivate or otherwise disable the product or products of the target insect gene or genes. A second consequence of the genomic revolution has been the development of molecular methods to investigate the composition and function of microbial communities, including unculturable forms, leading to the recognition that the sustained vigor and fitness of both plants and insects are dependent on interactions with resident microorganisms, collectively known as the microbiome (Douglas, 2015). Briefly, the Cas9 protein is a DNA-specific nuclease that makes a double-stranded break in DNA at a site guided by the binding of a synthetic guide RNA. Multiple CRISPR protocols are available, including those with the capacity to generate site-specific indels (often yielding frameshift mutations), to replace or insert specific sequences, and (by using a deactivated Cas9) to suppress gene expression. In relation to insect pests, the first applications of CRISPR in crops confer resistance to insect-vectored viruses, especially the geminiviruses, which have DNA genomes (Fondong, 2017). CRISPR is also the technology of choice to produce new crop varieties in response to insect pest genotypes that break plant resistance mechanisms. This is because resistant and susceptible alleles of plant resistance loci generally differ by just one or a few nucleotides. Specifically, CRISPR can be used to edit the susceptible allele to the resistance allele, thereby eliminating the need for extensive crosses and back crosses by conventional methods. The relative ease with which CRISPR can be applied to edit all copies of a gene makes CRISPR the technology of choice for polyploid crops. It is becoming increasingly evident that members of the microbiome can influence insect-plant interactions and can contribute to strategies for enhanced crop resistance to insect pests.

RNA interference (RNAi)

Alternatively, other strategies like RNA interference (RNAi) aimed at silencing of selected genes involved in insect feeding. Either as an alternative or a complement to Bt toxins, RNA interference (RNAi) has great promise for insect pest control (Ren et al., 2019). RNA Interference RNA interference (RNAi) offers the opportunity to design insecticides that have even greater flexibility than protein toxins with regard to both mode of action and specificity. Double-stranded RNA (dsRNA) specific to an essential gene of an insect pest is internalized into cells, where it is processed by Dicer enzymes to small interfering RNA (siRNA) molecules that guide the Argonaute protein of the RNA-induced silencing complex (RISC) to degrade complementary mRNAs and, in some instances, to interfere with translation of the target mRNA (Scott et al., 2013). RNAi can therefore be exploited to suppress gene expression through highly specific depletion of target transcripts. The functional RNAi machinery has two major components, (1) the core component inside the cells, which is comprised of Dicer enzymes, RNA-binding factors, and Argonaute protein, and (2) systemic component that amplifies the dsRNA signal and allows it to spread to other tissues within the animal (Siomi & Siomi, 2009). dsRNA is most commonly delivered by genetic modification of the plant (Price and Gatehouse, 2008), but topical application of dsRNA by sprays or drenches has also been reported to control lepidopteran and hemipteran pests (Li et al., 2015). Orally delivered RNAi is particularly effective against many coleopteran insects, routinely mediating >80% reduction in expression of target genes and conferring significant crop protection, e.g., in corn against the western corn rootworm (Baum et al., 2006), and in potato against the Colorado potato beetle Leptinotarsa decemlineata (Zhang et al., 2015). Besides oral delivery, dsRNA constructs can be administered in insects topically, through soaking and with microinjections into hemolymph (Yu et al., 2013). Microinjections can bypass the midgut, thereby inducing a systemic response. There are several microinjection techniques available, but the majority of them are time consuming and require equipment ranging from in-house produced devices to sophisticated microprocessor-controlled injectors (Dzitoyeva et al., 2001). Thus, microinjection procedures are not practical as means of pest control, but they are useful to investigate optimal dsRNA candidates and to demonstrate proof of concept. Topical administration is defined as direct dsRNA administration via the exoskeleton. It can be achieved by uniform spraying of dsRNA in the whole insect body (Wang et al., 2011) or through ventral micro-application. Relative to injections, this would be labor-saving and can allow for high-throughput gene screening. However, only a few publications have shown promising results using this approach (Killiny et al., 2014). One of the few examples includes studies conducted in adult *Diaphorina citri*, in which dsRNA solutions targeting five *cytochrome P450* genes was applied topically into the thoracic region. Mortality was significantly higher in adults treated with dsRNA than untreated controls. A similar method has also been described for *Ostrinia nubilalis* larvae, in which topically applied fluorescent dsRNA confirmed that dsRNA did penetrate the body wall and circulate in the body cavity (Wang et al., 2011). RNAi against lepidopteran and hemipteran pests is used widely in research but can be less reliable than in the Coleoptera (Scott et al., 2013). Strategies to enhance the efficacy of in planta RNAi against insect pests include expression of long hairpin RNA (hpRNA) in the chloroplast to minimize processing by the plant RNAi machinery (Bally et al., 2016) and stacking the hpRNA against the gene of interest with hpRNA against nonspecific nucleases expressed in the gut of the target insect (Song et al., 2017).

Gene pyramiding

Resistance developed through a single gene can be overcome by pests after a few years (Esse et al., 2020), so it is necessary to develop unique and efficient strategies to enhance crop resistance against stresses to improve yield and quality on a sustainable basis (Zafar et al., 2020a). Gene pyramiding may be one of the superior techniques to accomplish durable resistance against various stresses in crop production (Razzaq et al., 2021). Sustainable improvement of crops by integrating multiple resistance genes is essential to ensure agricultural production across a range of climatic conditions (Ren et al., 2019; Zafar et al., 2020a). In most cases, more than one gene controls a specific trait, so it is necessary to manipulate multiple genes for evolving resistance against biological and non-biological agents, such as chemicals, diseases, pests, and weeds (Razzaq et al., 2021). For long-term and durable resistance development, the pyramiding of diverse resistance genes against a single pathogen or pest in a single genotype can help for long-term resistance development (Nelson et al., 2018). Marker-assisted breeding could make it possible to effectively combine resistant genes into a single genetic background in the shortest possible time (Dixit et al., 2020).

Multiple Gene Pyramiding and Silencing (MGPS)

Insect pests can acquire resistance against single Bt toxins; therefore, pyramided Bt crops and efficacy of refuge for regulating the evolution of resistance against Bt-crops were introduced to overcome this resistance (Carrière et al. 2019). Recently, studies have suggested that insect pests (i.e., *P. gossypiella, H. zea, S. frugiperda*) have developed tolerance against dual gene pyramided cotton, and refuge also lost its efficacy in case of non-recessive resistance, i.e., cotton bollworm (Jin et al. 2015). Presently, new strategies are needed to be developed to delay the evolution of resistance in agricultural pests. Plant-mediated RNAi of essential pest genes involved in defense, detoxification, digestion and development is being utilized for enhancing tolerance against insects and pests. In recent years, new types of insect resistant transgenic crops have been developed using RNAi technology or RNAi pyramided with Bt genes (Ni et al. 2017; Zafar et al., 2020a). Ni et al. (2017) developed a pyramid of cotton containing Bt and RNAi, and found excellent results against cotton bollworm, but also substantially delayed resistance as compare with using Bt alone. Pyramiding of multiple RNAi expression cassettes against various essential genes involved in defense, detoxification, digestion and development of agricultural pests will successfully obtain favorable agronomic characters for crop protection and production. The MGPS involves the construction of transformable synthetic chromosomes, that have multiple distinct Bt toxins and RNAi to knockdown various essential target genes of pest (Ren et al. 2019). The evolution of resistance in agricultural pests will be delayed or blocked due to synergistic action of high dose of Bt toxins and RNAi(s) as well as compliance of ample refuge. The transgenic crops based on MGPS coupled with refuge can be an effective and smart way to control pests.

Conclusions

Currently, different strategies like pyramided crops expressing two or more distinct genes, refuge strategy and genome editing by CRISPR/Cas9 and RNAi are being used to control insect pests. The insects are remarkably adaptable and can develop resistance to any control tactics, including transgenic plants containing multiple Bt toxins and RNAi. The innovations like genetically modified Bt toxins and discovery of insecticidal proteins from bacteria other than Bt will continue to provide new tools for pest control. The MGPS-based crops will be more durable with compliance of high refuges and other control tactics.

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