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Spotlight

iJAZ: the next breakthrough for engineering pest-resistance in plants?

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Although transgenic *Bacillus thuringiensis* (Bt) crops have brought various ecological and socioeconomic benefits, there is evidence suggesting that pests will eventually develop resistance to Bt crops. Thus, additional genes are urgently needed to engineer pest resistance in plants. A recent study by Mo *et al.* indicates that iJAZ maybe the next breakthrough for engineering pest resistance in plants.

Plants urgently need more reliable genes for coping with pest infections

Pests have a significant impact on plant growth, development, yield, and quality. According to the Food and Agriculture Organization (FAO), pests cause an annual loss of 20–40% in global crop production, valued at approximately US$70 billion. To battle pest infection and damage, academic and industrial communities have developed different strategies. Among these strategies, transgenic plants (primarily Bt crops) have been the most successful. In these plants, foreign genes are inserted into plant genomes to produce specific biopesticide proteins that target certain insects. Since their initial adoption by farmers in the early of 1990s, Bt crops have become a crucial tool in pest control. Over the past two decades, Bt crops have been cultivated in over 100 million hectares worldwide [1], providing numerous ecological and socioeconomic benefits. However, Bt crops are currently facing a significant challenge. Laboratory and field studies have shown that pests have slowly developed resistance to Bt proteins, ultimately leading to the failure of pest resistance [2,3]. Thus, there is an urgent need for identifying additional genes for engineering pest-resistance in plants.

JAZ24 is a naturally inherited insect-resistant gene with dual functions

JAZ24 is a jasmonate (JA) ZIM-domain protein that is highly conserved in the plant kingdom, particularly in angiosperms. By chance, researchers discovered that JAZ24 has dual functions [4]. When JAZ24 is overexpressed, it leads to sterility in transgenic cotton plants, but it also provides resistance to both cotton bollworm and fall armyworms (FAW) [4].

JAZ24 is an atypical JA-sensitive JAZ protein, in which the canonical LPIAR (Leu–Pro–Ile–Ala–Arg) loop region of the degron was replaced by TSLSM (Thr–Ser–Leu–Ser–Met). Thus, JAZ24 does not show the jasmonoyl-isoleucine (JA-Ile)-dependent interaction with COI1 [the F-box subunit of an SCF (SKP1/Cullin1/F-box)-type ubiquitin E3 ligase) [4]. However, GhJAZ24 contains an ethylene response factor (ERF)-associated amphiphilic repression (EAR) motif, which binds to co-repressors (TOPLESS and HDA6) to regulate plant fertility [5,6]. This is a dosage-dependent function; plants show fertility only when JAZ24 is overexpressed (Figure 1A).

JAZ24: insecticidal mechanism

A recent study conducted by Mo and colleagues found that transgenic plants overexpressing JAZ24 were lethal to FAW, an important lepidopteran pest which damages over 350 plant species belonging to 76 families, including many important crops, such as cotton and corn [4]. Further investigations showed that JAZ24 confers toxicity and/or plant resistance not only towards FAW but also towards other important pests, such as bollworm, in a concentration-dependent manner similar to that of Bt proteins. However, the action model of JAZ24 differs from that of Bt proteins. GhJAZ24 contains an NGR peptide motif, which first recognizes and then binds to the aminopeptidase N (APN) receptor [7], found on the membrane of pest endothelial cells. This binding allows the JAZ24 protein (possibly by forming a channel) to enter into pest GI endothelial cells, disrupt histone deacetylase 2 (HDAC2), induce cell inflammation, and ultimately cause cell death. Among several APN receptors in pest cells, GhJAZ24 binds strongly to SfAPN4 with a KD(M) of 2.38×10^{-7} similar to the KD(M) (1.10×10^{-7}) of CryAc–APN interaction [4]. Although both Bt and JAZ24 utilize APN receptors to enter pest cells, they recognize and bind different regions; GhJAZ24 binds to the APN NHP domain (345N–472Y), whereas Cry1AC binds to the 949T–970T region of APN1 [8]. This distinct binding mechanism allows GhJAZ24 and Bt to function independently against pest infections, potentially prolonging the effectiveness of transgenic pest-resistant crops. Bioassay analysis in sf9 cells showed that GhJAZ24 induced cell division arrest at the G2/M phase and caused apoptosis and cell death with an IC50 value (value in which the growth of 50% of the cells were inhibited) of approximately 0.11 μM; the data suggest that low concentrations of GhJAZ24 can lead to arrest of cell growth and can even cause cell death [4]. Although other NGR-containing JAZ proteins also exhibit resistance to certain pests, GhJAZ24 demonstrated the highest insecticidal activities with the greatest efficacy [4]. The NGR motif is required to allow JAZ24 binding to APN, as mutations in this motif, such as GhJAZ24^{LGK}, led to a loss of JAZ24 binding activity to any APN proteins in pest cells and reduced the toxicity of JAZ24 to Sf9 cells and FAW [4].

iJAZ serves as a biotechnological tool against pests for environmental sustainability

GhJAZ24 is a plant-derived insecticidal protein which effectively eliminates various agriculturally important pests at low dosages

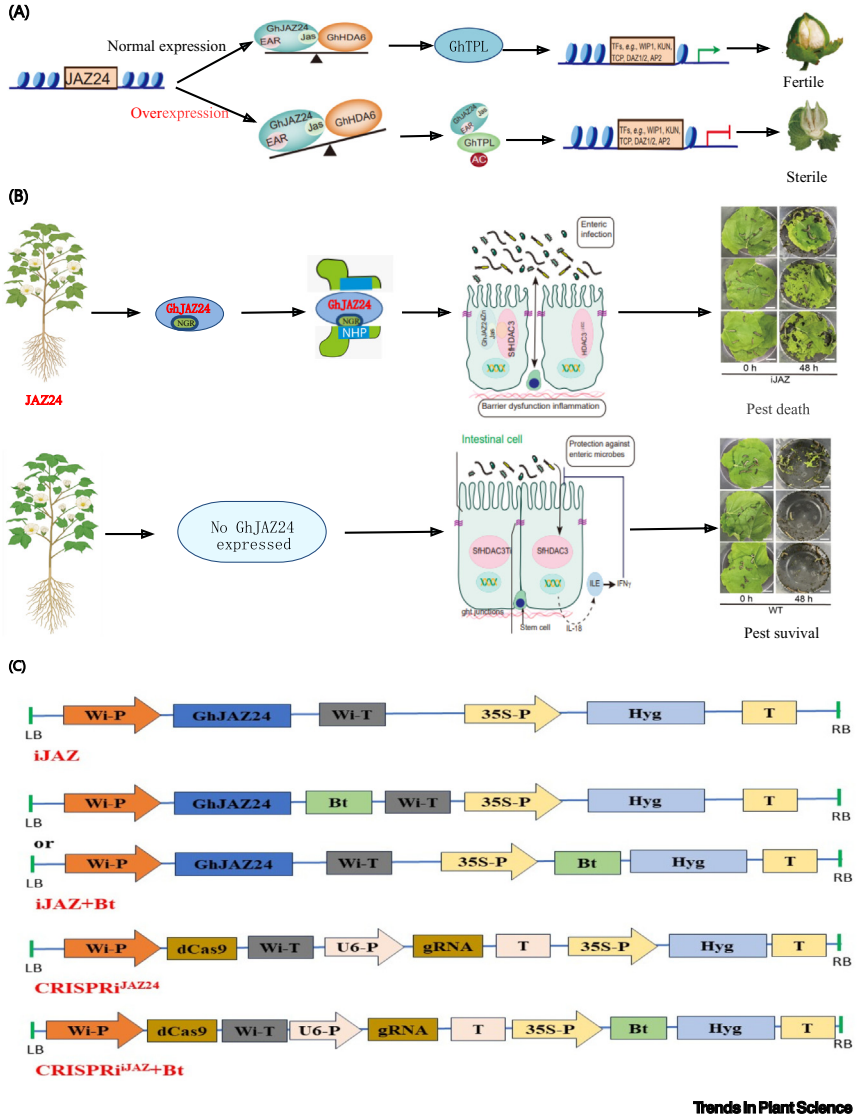

Figure 1. The dual function of GhJAZ24 and its potential application for creating transgenic plants for pest resistance. (A) JAZ24 regulates plant fertility. JAZ24 proteins contain an ethylene response factor (ERF)-associated amphiphilic repression (EAR) motif that recruits the Groucho/TUP1 (chromatin-silencing transcriptional regulator)-like transcriptional repressor Topless (TPL). TPL binds to and regulates the expression of different transcription factors (such as WIPI, KUN, TCP, AP2, and DAZ1/2) to control plant fertility [6]. (B) JAZ24 is a naturally plant-derived insecticide protein that targets various pests. The JAZ24 protein enters pest gastrointestinal (GI) tract cells through the interaction of JAZ24 NGR motif with the NHP domain of the membrane receptor APN4, leading to pest-cell apoptosis and degradation. The action mechanism differs from that of *Bacillus thuringiensis* (Bt) proteins [12], making JAZ24 more useful in engineering pest resistance in plants. The transgenic plants with overexpression of JAZ24 caused pest death after 48 h; however, the pests grew well when they fed on the non-transgenic plant leaves. (C) The major transgenic and clustered regularly interspaced short palindromic repeats (CRISPR) genome editing strategies for using JAZ24 to control agriculturally important pests in an environmentally sustainable manner. Both traditional transgenic methods and advanced CRISPR/Cas genome-editing technology can be employed to create JAZ24 plants for pest control. To enhance suitability of JAZ24 for pest control, wound-induced promotors (Wi-P) and terminators (Wi-T) – such as pAtLOX2, AtLOX2, AtLOX2, and pAtVSP1 – are suggested to be used for JAZ24 gene. JAZ24 and Bt proteins can be combined to enhance the pest resistance of

(Figure legend continued at the bottom of the next page.)

across multiple plant species, offering potential for developing advanced pest-resistant crops through biotechnological methods. However, due to its dual functions, its application has some challenges. To solve this problem, Mo and colleagues designed an iJAZ approach to effectively target infected pests without affecting plant fertility. The wild-type GhJAZ24 protein contains a nuclear localization sequence (NLS) (KRKTRIQAT) that localizes it in the nucleus, recruiting TOPLESS and HDAC6 to cause plant sterility [6]. Dysfunctional NSL sequences reduced its negative effect on plant fertility [4], but also reduced the toxicity of GhJAZ24 against pests, such as FAW. To overcome this issue, the authors added the signal peptide sequences (AtPR1, AtPR3, AtPR5, and AtPDF1.2) targeting the outer membrane to the N terminus of GhJAZ24. This modification facilitated the translocation of overexpressed GhJAZ24 proteins from intracellular to extracellular locations, enhancing plant resistance to pests without compromising plant fertility. To improve the efficiency of transgenic plants against pests, an iJAZ module was developed to regulate GhJAZ24 expression. This module consisted of a synthetic gene circuit with four GhJAZ24 tandem expression cassettes controlled by damage-induced promoters (pAtLOX2, AtLOX2, AtLOX2, and pAtVSP1) to regulate GhJAZ24 expression. Additionally, JAZ24 codons were modified to avoid potential gene silencing or homologous recombination caused by the transgenic copies [9]. These changes led to the induction of GhJAZ24 expression, reaching peak levels at 5 h after pest-induced damage in transgenic plants, effectively targeting and eliminating FAW and bollworms without affecting other important agronomic traits [4]. The iJAZ strategy was also successfully introduced into other crops, including rice (*Oryza sativa*), maize (*Zea mays*), and tobacco (*Nicotiana tabacum*), where GhJAZ24 expression conferred resistance to various additional pests, especially lepidopteran pests, such as *Ostrinia nubilalis* and *Plutella xylostella*. Bioinformatic analysis demonstrated that the NHP domains of APN and

the Zn-binding site domain of HDAC3 were highly conserved in many herbivorous insects, especially in lepidopteran pests, suggesting that JAZ24 has broad-spectrum insecticide activity suitable for combating numerous lepidopteran pests [4].

Concluding remarks and future perspectives

JAZ24 has shown the potential for controlling multiple agriculturally important pests in a variety of crops [4]. By comparison with Bt proteins, JAZ24 kills pests through a distinctive action mechanism. This allows for JAZ24 not only to be used for the generation of transgenic JAZ24 plants but also to be combined with Bt to generate transgenic plants for multiple pest resistance. Numerous experimental and bioinformatics analyses show that combing multiple pest-resistant genes with different action mechanisms significantly enhanced not only the lifespan but also the pest resistance of transgenic crops from 7–10 years to 20–30 years [10]. Bt and JAZ24 are another example of this synergistic effect, enhancing each other's efficacy. The application of JAZ24 will increase the usage of Bt crops, leading to increased farmer income through higher yields and reduced pesticide usage. Thus, the combination of JAZ24 and Bt may represent the future of transgenic plants for pest resistance, contributing to sustainable agricultural development and food security (Figure 1C).

JAZ24 is a naturally occurring plant gene that is widely expressed in plants. Despite its prevalence and the dual function found for fertility and pest resistance, its function is still not fully understood. Beyond these two functions, JAZ24 may also affect other traits. If not, this gene could have broad applications, such as in vegetative and ornamental crops. In these cases, JAZ24 could be directly overexpressed to enhance total expression levels, improving plant protection against pests. Additionally, utilizing transgenic plants with sterility induced by JAZ24 could reduce pollen and/or seed impact on air quality, and may also enhance vegetative growth and plant biomass. JAZ24 can also be regulated using clustered regularly interspaced short palindromic repeats inducer (CRISPRi) technology [11], where CRISPR/deactivated Cas9 (dCas9) can be combined with an inducer to enhance JAZ24 expression under a wound-induced promotor (Figure 1C). CRISPRi-mediated gene regulation has been used in plants to induce and/or repress gene expression and further control individual phenotypes [11]; one of the main advantages of CRISPRi is to pool guide RNAs (gRNAs) to boost the expression of an individual gene and also to potentially target multiple gene members in the same gene family, such as transcription factors that are crucial for plant growth and development, and response to various environmental stresses. Wound-induced regulation of JAZ24 also avoids the potential negative effects of plant sterility because JAZ24 is highly expressed only during insect infestation without affecting normal plant growth and development. Thus, the use of CRISPR/Cas genome-editing technology could enhance the effectiveness of JAZ24 and other insecticidal genes and/or proteins in pest control.

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Declaration of interests

The authors declare no conflicts of interest.

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transgenic plants, in which both JAZ24 and Bt can share the same promotors (Wi-P) or use different promotors, such as CaMV-35(35S-P) for consistent expression of Bt protein. CRISPR inducer (CRISPRi) technology can also be employed to enhance JAZ24 expression by fusing deactivated Cas9 (dCas9) protein (under the Wi-P promotor) with an inducer (guide RNA, gRNA) for a JAZ gene. When pests infect CRISPRi-modified plants, the wounding response induces the expression of JAZ24 which then triggers the response against the invading pests. For this strategy, a specific gRNA targets a specific JAZ gene.