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Biotechnology Congress 2015 : Silencing of hormonal biosynthesis genes by double stranded RNA (dsRNA) impairs larval growth and development of cotton bollworm (Helicoverpa armigera) - Anjali Jaiwal - University of Delhi

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Helicoverpa armigera is a polyphagous insect pest responsible for major losses in cotton and other agronomically important crops. RNA interference (RNAi) has emerged as a potential alternative to raise insect-resistant plants by in planta expression of dsRNA specific to a vital insect gene. In the present study, the hormonal biosynthesis genes in H. armigera were targeted by feeding dsRNAs corresponding to each target gene viz. Juvenile Hormone Acid Methyl Transfererase (HaJHAMT), Pro-Thoracicotropic Hormone (HaPTTH), Pheromone Biosynthesis-Activating Peptide (HaPBAP), Molt Regulating Transcription Factor (HaHR3), Activated Protein 4 (HaAP-4) and Eclosion Hormone Precursor (HaEHP) which play key roles in regulation of physiological, developmental and behavioural events in the target insect pest. Ingestion of target gene dsRNAs via artificial diet resulted in variable mortality ranging from 60-92% in all the six targeted genes.

Silencing of the target genes showed retarded larval growth, delayed in molting, metamorphosis and pupal formation. A comparison of the silencing potency of un-diced long HaPTTH dsRNA with RNase III-diced-siRNAs revealed that long dsRNAs were more effective in target gene silencing as compared to siRNAs. The HaPTTH-dsRNA coated onto the detached leaf was found to be more effective in silencing target gene when compared to dsRNA feeding via artificial diet. The qRT-PCR analyses showed that mRNA level of six target genes was drastically reduced compared to control or unrelated GFP-dsRNA control correlated with the developmental defects. These results indicate that hormonal biosynthesis genes can be used as vital targets for improving pest resistance in cotton and other crop plants which are infested with H. armigera. RNA impedance (RNAi) has been created as a ground-breaking procedure in the exploration of utilitarian genomics just as plant bother control. In this report, twofold abandoned RNAs (dsRNA) focusing on 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) quality, which catalyze a rate-restricting enzymatic response in the mevalonate pathway of adolescent hormone (JH) amalgamation in cotton bollworm, was communicated in cotton plants by means of Agrobacterium tumefaciens-interceded change. PCR and Sothern investigation uncovered the coordination of HMGR quality into cotton genome. RT-PCR and qRT-PCR affirmed the high translation level of dsHMGR in transgenic cotton lines. The HMGR articulation both in interpretation and interpretation level was altogether downregulated in cotton bollworms (helicoverpa armigera) hatchlings subsequent to benefiting from the leaves of HMGR transgenic plants. The interpretation level of HMGR quality in hatchlings raised on transgenic cotton leaves was as much as 80.68% lower than that of wild sort. What's more, the relative articulation level of vitellogenin (Vg, vital wellspring of sustenance for posterity undeveloped organism improvement) quality was likewise diminished by 76.86% when the bug hatchlings were taken care of with transgenic leaves. The aftereffect of bug bioassays demonstrated that the transgenic plant harboring dsHMGR restrained net weight gain as well as postponed the development of cotton bollworm hatchlings. Taken together, transgenic cotton plant communicating dsRNAs effectively downregulated HMGR quality and hindered the turn of events and endurance of target creepy crawly, which gave more alternative to plant bug control.

Catchphrases: 3-hydroxy-3-methylglutaryl coenzyme A reductase(HMGR), cotton bollworm, RNA obstruction, transgenic cotton, twofold abandoned RNAs, bother control. Cotton (Gossypium hirsutum) is a significant fiber and monetary yield far and wide, which shows prominent essentialness in crop creation. Nuisances and pathogenic organisms present principle worry for the profitability and nature of cotton. Right now, the significant irritation in cotton creation is cotton bollworm (Helicoverpa armigera). Despite the wide development of transgenic creepy crawly safe BT cotton indicating huge financial and social superiorities 1, the transformations of bollworm quality among ages and the choices coming about because of BT bug safe proteins bless bollworm with protection from transgenic BT crops 2-6. Henceforth transgenic creepy crawly obstruction crops with elective procedures is alluring. RNA obstruction (RNAi), a viable quality quieting instrument in eukaryotes, has been found in Caenorhabditis elegans 7 just because and afterward created as a successful bug safe framework in a wide assortment of plant species 8, 9. Twofold strand RNA (dsRNA) can be created by inside translation, transposon, fake transgenesis and RNA infection contamination, which are perceived and

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deteriorated into little meddling RNAs (siRNA) by endoribonuclease Dicer. RNA-prompted hushing complex (RISC), including siRNA and a few chemicals, for example, endonucleases, exonucleases and helicases, displays the capacity of nucleases to perceive and separate explicit objective RNA 10. Along these lines, RNAi can be misleadingly used to restrain the outflow of endogenous quality. Sharing normal atomic instrument of arrangement explicit quality quieting in a wide assortment of animal varieties, RNAi activated by exogenous dsRNA has been created as one of the most productive apparatuses for the exploration of quality capacity 11as well as nuisance control. The dsRNA created by transgenic plants against key quality of irritations has been viewed as shield that enriches transgenic safe vermin plants with new advancement. Cotton bollworm experiences complex shedding process in its life cycle, whose development and improvement are coordinately controlled by hydroxyecdysone and adolescent hormone (JH). Analogs of JH have been integrated falsely and carried new originalities to the essential innovative work of pesticide. Fruitful uses of hormone analogs identified with shedding process demonstrated that impedance or exaggerating of pivotal hormones could be another methodology for the coordinated bug the board (IPM) of creepy crawlies having a place with the Phylum Arthropoda. Along these lines, the critical qualities or interpretation factors associated with the hormone biosynthesis pathways are slanted to be utilized as perfect targets when RNAi innovation is applied to bug control. JH is integrated through the mevalonate pathway in creepy crawlies, in which mevalonate is one of the most significant intermediates. HMG-CoA, the antecedent of mevalonate pathway, is obliged to experience three enzymatic responses to be changed over into mevalonate, and HMGR catalyzes and manages the last response 26. In this manner, HMGR connects a rate-constraining advance in the biosynthesis of mevalonate, rising as a promising objective for the RNAi innovation for creepy crawly control 27, 28. Furthermore, the biosynthesis of vitellogenin, the significant sustenance for posterity undeveloped organism advancement, likewise can be hindered by the downregulation of HMGR quality in B.germanica 27. In our past report, a 3329-bp full-length cDNA (GenBank increase no. GU584103) of HaHMGR quality has been cloned by RACE innovation. A 1176-bp section in the coding arrangement of HaHMGR was intensified from the cDNA of cotton bollworm and used to create dsRNA. By infusing dsHMGR into the midsection of 2-day-old pupa, we saw that the quantity of eggs laid was diminished and the overall articulation of both HMGR and Vg quality was downregulated in the tried hatchlings.

Biography

Anjali Jaiwal is pursuing PhD under the supervision of Professor M V Rajam, Head of Department of Genetics, University of Delhi South Campus, New Delhi, India. She did her Post-Graduation in Biotechnology from M D University, Rohtak, Haryana and received Gold Medal for standing first in the university. She received different scholarships during Graduation and Post-graduation. She cloned and submitted three gene sequences to GenBank of NCBI. She published one review article ‘Coenzyme Q10 production in plants: current status and future prospects’ in ‘Critical Reviews in Biotechnology’ as second author. She attended six national and three international conferences. She was awarded CSIR-UGC JRF and prestigious DST-INSPIRE Fellowship by the DST (Department of Science and Technology). Her research work includes the screening of few vital genes of Helicoverpa armigera by feeding target gene dsRNAs to the insect pest via semisynthetic artificial diet and the development of insect resistant transgenic tobacco and cotton plants via plant-mediated RNAi silencing of vital genes of H. armigera.

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