MEETING REPORT

Basic research to herald a new era in pest management science*

We have been battling against arthropod pests ever since agriculture began. Chemicals have been used as the main weapons against the pests[1]. The use of chemicals to suppress arthropod pests can be traced back to at least 4000 years. Chemicals gave humans an unprecedented manipulative power to suppress pest populations and protect crops. The synthetic chemicals were considered a panacea for all pest problems in agriculture and public health. Insecticides with different modes of action, different formulations, delivery systems, pheromones, kairomones and semiochemicals are also being used to fight insects[2]. Soon the harmful effects of pesticides came to light, popularized by the publication of *Silent Spring* by Richael Carson[3]. Alternative options for pest control were initiated. Research stimulated development of non-chemicals methods, including the use of pest-resistant varieties and genetically modified crops, male sterile technique, biological control, cultural and mechanical, physical methods, push–pull strategy, insect growth regulators and semiochemicals[4]. However, there is an urgent need to look for an effective and safer alternative, where RNA interference (RNAi) comes handy. RNAi, a sequence-specific gene silencing mechanism has immense potential in agriculture for pest management. Consequently, a workshop on insect physiology, toxicology and RNAi for pest management was organized, where Subba Reddy Palli (University of Kentucky, USA), delivered a series of lectures. Delegates from ICAR institutes, State Agricultural Universities (SAUs), scholars, faculty members and students participated in the deliberations.

The session began with Palli's lectures that mainly focused on hormonal regulation of gene expression in insect pests with a goal to identify proteins involved in signal transduction of ecdysteroid, juvenile hormones (JHs), and to use them for developing novel, environmentally-safe pest management methods. Lectures delivered were: 'Role of next generation technologies in pest management: approaches and opportunities'; 'Role of RNAi in pest management'; 'Applications of genomics and functional genomics technologies in insecticide resistance management'; 'GM crops in pest management: challenges and solutions' and 'Interactive session on changes in the horizon for entomology: exciting opportunities for students and early career scientists'.

RNAi is a mechanism of gene silencing triggered by double-stranded RNA (dsRNA). It was discovered in the nematode worm, *Caenorhabditis elegans* by Andrew Fire and Craig C. Mello. They shared the 2006 Noble Prize for Physiology or Medicine for their work on RNAi, which they published in 1998. Within years of this discovery, it was shown to function in nematodes, insects, humans and plants. This was followed by refinements in design, synthesis and delivery of dsRNA or small interfering RNA (SiRNA) that led to the development of RNAi applications in both human and plant health. Gene silencing is achieved after dsRNA or SiRNA molecules bind to complimentary DNA/RNA interfering with translation, transcription or replication of the target gene. The target DNA/RNA to be silenced should have 100% identity for efficient recognition and subsequent interference. Therefore, unlike other methods of pest control, RNAi is highly specific to target species.

The RNAi pathway is initiated by the enzyme Dicer, which cleaves long double-stranded RNA (dsRNA) molecules into short double stranded fragments of 21–23 nucleotide SiRNAs. Each SiRNA is unwound into two single-stranded RNAs (ssRNAi) – the passenger strand and the guide strand. The passenger strand is degraded and the guide strand is incorporated into the RNA-induced silencing complex (RISC). The well-studied outcome is post-transcriptional gene silencing, which occurs when the guide strand pairs with a complementary sequence in a messenger RNA molecule and induces cleavage by Argonaute protein (AGO), the catalytic component of the RISC complex. The pathway is used as a tool in medicine and agriculture. RNAi is also exploited to understand the regulation of the male and female reproduction. The event of reproduction is precisely regulated by multiple pathways, including ecdysone, JH and nutrition. One such application is to make sterile insects[5–8].

According to Palli, RNAi works well in some insects, especially those that belong to order Coleoptera (beetles). It does not seem to work well in insects that belong to order Lepidoptera (moths and butterflies). Extensive studies in coleopteran insects such as in the red flour beetle, *Tribolium castaneum* Herbst, a pest of stored products and the western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte and the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) that attack crops grown on smaller farms and greenhouses showed the utility of RNAi in both basic and applied sciences. Small quantities of dsRNA appear to be sufficient to initiate RNAi response in these insects.

Research conducted in Palli's laboratory showed the involvement of JH and ecdysteroid signalling in the regulation of female reproduction in *T. castaneum.* JH regulates vitellogenin (Vg) synthesis in the fat body. Palli reported ecdysteroid regulation of ovarian growth and oocyte maturation. Microarray analysis of RNA isolated from ovaries showed the upregulation of several genes coding for proteins involved in ecdysteroid signalling on the fourth day after female adult eclosion. The functional analyses of genes coding for proteins involved in ecdysteroid and JH regulate ovarian growth and primary oocyte maturation. Ultrastructural studies showed the temporal sequences of key events in oogenesis, including the development of primary oocytes, differentiation and development of follicle epithelial cells, and formation of intercellular space to facilitate uptake of Vg protein. RNAi studies showed that

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ecdysone receptor (EcR) and ultra spiracle (USP) are required for the ovarian growth, primary oocyte maturation and growth and migration of the follicle cells. These studies suggest important roles for ecdysteroids in the regulation of oocyte maturation in the beetle ovaries[9–12].

One of Palli's studies identified JH and nutrition as the two key signals that regulate *Vg* gene expression in *T. castaneum*. JH regulation of Vg synthesis has been known for a long time in several insects, but the mechanism of action is not known. Experiments were conducted to determine the mechanism of action of these two signals in regulation of *Vg* gene expression. Injection of bovine insulin or FOXO double-stranded RNA into the previtellogenic, starved, or JH-deficient female adults increased Vg mRNA and protein levels, thereby implicating the pivotal role for insulin-like peptide (ILP) signalling in the regulation of *Vg* gene expression and possible crosstalk between JH and mediate silencing of genes coding for acid methyl transferase or methoprene-tolerant beetles decreased expression of genes coding for ILPs and influenced FOXO subcellular localization, resulting in the down-regulation of *Vg* gene expression. Furthermore, JH application to previtellogenic female beetles induced the expression of genes coding for ILP2 and ILP3, and induced *Vg* gene expression. FOXO protein expressed in baculovirus system binds to FOXO response element present in the *Vg* gene promoter. These data suggest that JH functions through ILP signalling pathway to regulate *Vg* gene expression[13].

Palli spoke on the role of RNAi in silencing insecticide resistance-associated genes in insects. Studies on insects such as Colorado potato beetle with high potential for resistance development showed that RNAi could be used to fight insecticide resistance in the field[14]. Insects have developed resistance to almost all insecticides introduced so far[15]. Therefore, there is no reason to believe that dsRNA is immune to resistance development by insects. Mutations to genes coding for proteins involved in dsRNA transport, processing, risk complex formation and other processes involved in RNAi pathway as well as mutations to dsRNA target genes are potential mechanisms of resistance development. Recent studies on WCR suggest that resistance to Snf7 dsRNA due to single nucleotide polymorphisms in the 240 nucleotide target sequence is highly improbable because potentially 221 SiRNAs could target *Snf7* gene; non-target studies on a number of insect species showed that DvSnf7 dsRNA showing a minimum of 3–21 nucleotide matches with the target gene is sufficient to silence target gene. Therefore, loss of some of the 221 SiRNAs due to mutation may not lead to significant levels of resistance. Future studies on the possible mechanisms of resistance development by insects against dsRNA would be valuable in developing dsRNA as a pesticide. Differences in efficacy of dsRNA on target pest could also aid in the evolution of resistance. In a recent study, evaluation of effects of RNAi treatments against immune gene *att1* on adults of three Western corn rootworm population exhibiting different levels of gut cysteine protease activity showed difference in RNAi efficiency among these three populations.

Palli spoke on 14 molecular markers associated with pyrethroid resistance in bed bug, *Cimex lectularius* Linn identified by transcriptome analysis. The resistance-associated genes functioning in diverse mechanisms are expressed in the epidermal layer of the integument, which could prevent or slow down the toxin from reaching the target sites on nerve cells, where an additional layer of resistance (kdr) is possible. The strategy evolved in bed bugs is based on their unique morphological, physiological and behavioural characteristics and has not been reported in any other insect species. RNA interference-aided knockdown of resistance associated genes showed the relative contribution of each mechanism towards overall resistance development. Understanding the complexity of adaptive strategies employed by bed bugs will help in designing the most effective and sustainable bed bug control mentors. NADPH-cytochrome P450 reductase (CPR) plays a central role in cytochrome P450 action. The genes coding for P450s are not yet fully identified in the bed bug, *C. lectularius*. Hence, cloning cDNA and down-regulating the expression of the gene coding for CPR is suggested to be required for the function of all P450s to determine whether or not they are involved in resistance of bed bugs to insecticides[16]. Palli's research has helped develop RNAi technology methods for suppressing insect pests and to fight insecticide resistance in beetles and bed bugs. Palli opined that RNAi gene silencing is successful in insecticide resistant arthropods where the existence of resistance mechanisms, viz. increased metabolism, decreased penetration, decreased transport and altered behaviour are involved. However, RNAi is futile in insect pests having resistance mechanisms such as target site insensitivity which reduce or eliminate the binding affinity of insecticides to their target proteins. New ideas are needed to manage resistance in such arthropods having multiple detoxifying mechanisms coupled with target site modifications.

Currently, RNAi technology can be used only for few insects and it is not commercially viable for many insects. So in future, efforts must be made to use RNAi technology against a wide range of insect pests. As technology is new, it is comparatively costly now. There are chances of resistance development by insects to RNAi technology. However, this technology should be safe. The progress will depend on how well the research community responds to existing needs and develops the necessary tools for production and application of RNAi-based insecticides in the field[17,18]. The landscape of insects science or entomology is rapidly changing. Some of the new challenges include bioterrorism, invasive species, diseases causing vectors, climate change, pollinator's health, insects as food sources and changing status of pests. Some of the new tools or methods that can be utilized in pest management science include gene sequencing advances in OMICS technology, RNA interference based functional genomics, genome editing methods, insect transgenic and genetic pest management.

RNAi has enormously contributed toward advances in functional genomics in insects. RNAi has great potential to contribute toward development of modern pest management methods. The major roadblocks in successful commercialization of dsRNA-based insecticides are: (a) lower efficiency in key pest species; (b) higher production and formulation costs; (c) lack of information about off-target and non-target effects and environmental fate; (d) unknown potential for resistance development, and (e) lack of thorough knowledge on mechanisms of action. Papers published during the past two years have made significant contributions toward eliminating some of these roadblocks and the studies during the next few years will probably increase our knowledge in these areas, resulting in successful commercialization of dsRNA-based insecticides.

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