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Genetic Improvement of *Trichogramma* Spp.

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SUMMARY

Genetic improvement of parasitoids and predators is an important trait in the field of biological control. It involves directed purposeful genetic alterations to enhance the efficacy of natural enemies of crop pests. Development of pesticide resistant predators and parasitoids helps in better integration and biological control and chemical control strategies, which are keystones of IPM. The genetic engineering approach may have potential for improving beneficial traits of natural enemies but variety of scientific, regulatory and political issues remain to be resolved before they can be used in practical pest management programs. The potential role of genetically modified natural enemies is likely to be explored within the foreseeable future. Agricultural ecosystems are artificial and selection or hybridization might be used to improve the effectiveness of arthropod natural enemies. Good amount of work on genetic improvement on Trichogramma has been done at different levels. An endosulfan tolerant strain of T. chilonis (Endogram) has been developed by Project Directorate of Biological Control (PDBC) Bangaluru for the first time in the world.

INTRODUCTION

Indiscriminate use of pesticides has led to serious concerns relating to their adverse effects on non-target organisms (Natural enemies), pesticide residues in food and food products, pest resurgence, development of resistance to insecticides, toxic effects on human beings, and environmental pollution (Orr, 2009). Widespread pesticide use has also led to elimination of natural enemies and, as a result, it has become practically difficult to control several insect species through the currently available chemical pesticides. Therefore, it is important to adopt pest control strategies that are ecologically sound, economically practical, and socially acceptable. In this context, development and deployment of natural enemies that are adapted to extremes of climatic conditions or are capable of tolerating sublethal doses of pesticides can play an important role in pest management. Recent, advances in molecular biology have broadened the available techniques for genetic manipulation of arthropods for a variety of traits in species of interest (Atkinson, Pinkerton, and O'Brochta, 2001). Release of genetically improved arthropods for suppression of pest populations has been undertaken in the past, and the possible applications of genetically modified arthropods have expanded considerably (Braig and Yan, 2002). Incorporation of foreign DNA into the genome has expanded the possibilities for genetic transformation of insects, although it has also raised a few questions.

The first genetically modified insects were produced 20 years ago with the restoration of wild-type eye color in a mutant strain of Drosophila melanogaster Meigen (Rubin and Spradling, 1982), followed by transformation of the Mediterranean fruit fly, Ceratitis capitata Weid. (Loukeris et al., 1995). Beneficial arthropods can be transformed for a variety of traits, and deployed more effectively as biocontrol agents. Some of the major constraints in using natural enemies in pest control are the difficulties involved in mass rearing and their ability to withstand adverse conditions. The molecular techniques can also be used to understand the genetics and physiology of reproduction and control of sex ratio, and this information can be used. The use of biological control in pest management systems has a long and rich history. Predators, parasitoids and pathogens have been successfully employed in many cases to control insect pests in an efficient, cost-effective and permanent manner. A variety of approaches have been in use to increase the performance of natural enemies. The traditional tactics viz., classical, augmentation and conservation have been vital and valuable tools. But emerging technique involving the genetic improvement of natural enemies of arthropods through selection, hybridization or recombinant DNA technology offers great promise (Beckendorf and Hoy, 1985). The genetic improvement of natural enemies aimed at developing superior natural enemies with specific traits like temperature tolerance, insecticide tolerance, increased virulence and amenability to mass production.

Trichogramma Spp.

Today, Trichogramma species are the most widely used insect natural enemy in the world partly because they are easy to mass rear and they attack many important crop insect pests. Nine species of Trichogramma are reared in private or government owned insectaries around the world and released annually on an estimated 80 million acres of agricultural crops and forests in 30 countries. The countries of the former Soviet Union lead in Trichogramma production, followed by China and Mexico. Trichogramma are released to control some 28 different caterpillar pests attacking corn, rice, sugarcane, cotton, vegetables, sugar beets, fruit trees and pine and spruce trees. Most releases are to control corn borers, sugarcane borers and cotton bollworm. Although widely used, a recent review of these programs worldwide concluded that “because of considerable variability in success of releases and little evidence of consistently successful application of Trichogramma, the usefulness of these parasitioids is currently being debated”.

Agricultural productivity in India is affected largely by insect pests and diseases, which cause losses to the tune of 10-30%. Though there has been increasing awareness in India about the hazards of indiscriminate use of pesticides in agriculture, use of biological agents for pest management has not taken off in a big way due to the susceptibility of bioagents to abiotic and pesticide-induced stresses, perceived slow action, lack of timely availability, etc. The efficacy of bioagents is affected by predisposing ecological factors, particularly temperature and habitat diversity, apart from host-plant interactions. Biocontrol agents are very susceptible to pesticides, though in a totally pesticide-free environment, they have been reported to be effective to the tune of 50-60%. Secondary pest outbreaks, pesticide resistance, more stringent pesticide regulation, and concern about human health and environmental quality have renewed the interest in Integrated Pest Management programs that emphasize biological control. The commercially successful use of Trichogramma to control the European corn borer in Europe has demonstrated the potential of this approach. Researchers in the U.S. are currently evaluating Trichogramma for the control of codling moth in apples and almonds, leaf rollers in apples, European corn borer in corn, and bollworm/budworm in cotton.

Taxonomy and Identification (Scientific Classification)

Kingdom: Animalia, Phylum: Arthropoda, Class: Insecta Order: Hymenoptera, Suborde:Apocrita Superfamily: Chalcidoidea, Family: Trichogrammatidae, Genus: Trichogramma. The genus Trichogramma is one of 80 genera in the family Trichogrammatidae. All members of this family are parasites of insect eggs. Trichogrammatidae includes the smallest of insects, ranging in size from 0.2 to 1.5 mm. Within the genus Trichogramma, there are 145 described species worldwide 30 species have been identified from North America and an estimated 20 to 30 species remain to be described. The species most commonly collected from crops and orchards are atopovirilia, brevicapillum, deion, exiguum, fuentesi, minutum, nubilale, platneri, pretiosum, and thalense. Trichogramma are difficult to identify because they are so small and have generally uniform morphological characters. Also, certain physical characteristics such as body color and the number and length of body hairs can vary with body size, season, rearing temperature and the host on which the adult was reared. Because of these difficulties and the lack of type specimens, species names in the literature in North America prior to 1968 were used incorrectly and inconsistently and are therefore unreliable (66). Further studies have shown that with the exception of the common species T. pretiosum, T. minutum and T. platneri, identifications of North American Trichogramma species published before 1980 are also largely unreliable.

Biology and Life Cycle

Trichogramma wasps primarily parasitize eggs of moths and butterflies (Lepidoptera). However, certain species of Trichogramma also parasitize eggs of beetles (Coleoptera), flies (Diptera), true bugs (Heteroptera), other wasps (Hymenoptera), and lacewings and their relatives (Neuroptera). The adult female wasp uses chemical and visual clues to locate a bollworm egg. The chemical clues, called kairomones, are on the moth scales left near the egg by the female moth during oviposition. Some of these same chemicals are also bollworm sex pheromones. Egg shape and color also may be visual clues to the wasp.

Life Cycle of Trichogrammatids:

Egg 1 day (16-24 hours), Larva 2-3 days, Pupa 4-5 days, Adult 2-4 days, Total life cycle 8-10 days

Target insects:
Sugarcane borers Chilo infuscatellus, Chilo sacchariphagus indicus, Chilo auricilius, Acigona steniellus;
Cotton (Non Bt) bollworms Helicoverpa armigera, Pectinophora gossypiella & Earias spp.;
Maize stem borer Chilo partellus,Diamond back moth Plutella xylostella;
Tomato fruit borer Helicoverpa armigera.

Selecting the Best Trichogramma species:

Different species and strains of Trichogramma typically prefer different host eggs and crop habitats and have different searching abilities and tolerance to weather conditions. Efficacy is improved by selecting the most effective and adapted species or strain for the specific crop/pest situation. Local species and strains collected from the target area and host often are the first choice for evaluation. However, exotic species and strains may be more effective and should be evaluated. If a species of interest is available commercially, it should be evaluated along with native species. However, unless the supplier maintains high quality control standards both genetic and product quality can deteriorate, leading to poor field performance.

Mass Rearing of Host Insect
(Corcyra cephalonica Stain.)

Procure sorghum/wheat / bajara / maize grains free from insecticides
↓
Grains heated/sterilized in an oven at 1000c for 30 min.
↓
Spray the grains with 0.1 % formalin
↓
Air dry grains to remove traces of formalin
↓
Grains milled to make 3-4 pieces of each grain using grinding mill
↓
Pour 2.5 kg of crushed grains in each wooden tray and add 10 g yeast
↓
Add 0.5 cc eggs of Corcyra and secure lid for 30 days.
↓
Observe boxes critically for emergence of adults after about 4 weeks
↓
Collect the adults emerged daily
↓
Keep adults in oviposition cage for egg laying
↓
Collect and clean deposited eggs and keep in glass vials with silica gel and plug with cotton
↓
Treat eggs with UV rays (30 W UV tube for 45 min. at a distance of 60 cm) to prevent hatching

Why need of improvement in Trichogramma:

Environmental hardiness, increased fecundity, improved host-seeking ability, Resistance to pesticides and modify the genome of natural enemies, Change sex ratio of parasitoids, Develop genetic linkage maps, Identify biotypes, Improve artificial diets, Altering sex ratio, shortening developmental rate.

Genetic Improvement of Trichogramma:

Genetic improvement of natural enemies involves directed and purposeful alteration in the genome of an organism (natural enemies) to enhance its efficacy as a biocontrol agent. Such genetic alteration can be achieved by artificial selection, hybridization and recombinant DNA technology. Identification and use of naturally occurring biotypes and maintenance of genetic quality is not genetic improvement. Genetically manipulated natural enemies could be used in Classical BC programs and Augmentative release.

Pesticide tolerance:

Pesticide resistance is inherited in simple Mendelian fashion indicating that it is a result of single gene mutation. Pesticide resistance is a monogenic trait. The pesticide tolerance trait is improved upon through artificial selection or genetic engineering. Pesticide resistant genes: Genes coding for enzymes (e.g: opd gene), Genes coding for pesticide targets (Ach or Na channel proteins), Genes coding for proteins affecting entry of insecticides are targeted for genetic improvement.

Environmental hardiness:

A major problem with parasitoid release is reduced survival or non-reproduction of the released parasitoid. This is particularly a problem with exotic parasitoid introduced into an area far from their natural range. For example, tropically adopted insects will probably not overwinter in temperate climates. Artificial selection and Genetic engineering is used for the improvement of this trait. The possible example is the genes responsible for Glycerol synthesis.

Diapause control:

To survive hostile seasonal weather, NE's undergo diapause. The predatory mites of greenhouse thrips undergo reproductive diapause in Europe during winter season. Artificial selection OR gene transfer is carried out to improve the diapauses trait. Identification of proteins, polypeptides, hormones regulating diapauses. Antisense genes used to turn off vital processes.

Disease resistance:

Disease is a common problem in laboratory & field. Disease affects competitiveness & survival in the wild after the release of natural enemies. Insects do not fight pathogens with the mammalian-type immunoglobulins. But in some insects, bacterial infection triggers the production of several different families of proteins having bacteriocidal activity.

Manipulation of genes for defensive proteins like cercopin, defensin or addition of new defensive gene could enhance the parasitoid response to microbial challenge.

Sex ratio manipulation:

Many mass rearing situations, it would be desirable to have an overabundance of one sex, usually female, since they parasitize the host. Female biased sex ratio is desirable in biocontrol. Sex ratio is regulated by host size and sex-ratio distorting factors. Three Extra-chromosomal factors are reported from Nasonia parasitoid. psr (daughterless) factor causes the production of all-male broods. msr factor skews the sex ratio toward females. sk (son-killer) factor, maternally inherited, causes production of females only.

Potential Risk Associated with Genetically Improved Biocontrol Agents:

Transgenic NE may pose risk to non-target insects: Genetically modified insect may replace the native insect due to its increased fitness.
Stability & inheritance of transgene.
Horizontal transfer of transgene & dominant selective markers. (antibiotic resistant genes)
Horizontal gene transfer through endosymbionts: Insects harbor many symboints, they may pick the trasgens and transfer to host insects.

CONCLUSION

Genetic manipulation of natural enemies of arthropods offers promise of enhancing their efficacy in pest management. Development of pesticide resistant predators and parasitoids helps in better integration and biological control and chemical control strategies, which are keystones of IPM. Genetic engineering of natural enemies has remained a controversial tactic in biological control, because most of the laboratory developed strains have not been tested in field condition and lack of regulatory mechanism, by which GM insects can be released into environment. However, to date only one transgenic arthropod natural enemy, a transgenic strain of the predatory mite, Metaseiulus occidentalis has been released on an experimental basis (McDermott and Hoy, 1997). Meanwhile, the genetic improved beneficial arthropods through artificial selection and hybridization continues to be explored in pest management. The genetic engineering approach may have potential for improving beneficial traits of natural enemies but variety of scientific, regulatory and political issues remain to be resolved before they can be used in practical pest management programs. The potential role of genetically modified natural enemies is likely to be explored within the foreseeable future.

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