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BIOOBJECTIFICATION: A NOVEL APPROACH IN IPM

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ABSTRACT

Sterile insect technique and inherited sterility are older methods through which insect-pests are used to be genetically modified without using biotechnological tools. Using biotechnology to modify genetic constitution of insect-pests in order to manage them is getting importance and popular now. Scientists are modifying insect-pests by inserting desired transgenes and use them to fight against their own wild counterparts to reduce their damage to agricultural crops as well as human beings which is called bio-objectification. A technique of bio-objectification, release of insects carrying a dominant lethal gene (RIDL) is being experimented and evaluated worldwide on different insect-pests to reduce their population and eventually damage. OX513A is a genetically modified strain of dengue mosquito which had successfully reduced wild mosquito population in open environment. Likewise, in agriculture, transgenic strains of diamondback moth, OX4319L and pink boll worm, OX3402C have also showed significantly appreciable results on controlling their wild insect population.

Key words: Bio-objectification, genetically modified organism, genetic engineering, release of insects carrying a dominant lethal gene, pest management, mosquito, diamondback moth, pink bollworm, resistance management

World's total human population is about 7.87 billion in the 2021. India is second largest populous country in the world but will take over China around the year 2027 (UNFPA, 2021; United Nations, 2019). Each and every living organism requires food for sustaining its life for which human beings and many other living entities depend upon crop plants. The only source of food is agriculture and India's 58% population depends on agriculture (APEDA, 2021). In agriculture, crop protection is an important aspect for higher and qualitative food production as there are varieties of pests including insects, pathogens and nematodes that are known for invading plant system. Since earlier, several management strategies have been utilised to control these insect pests.

Bioobjectification

The modification of genetic constitution of any living organism by any way in order to make them useful to human beings is called bioobjectification. It can be defined as the ranges of experimental work which are devoted to the exploration and fashioning of new forms of life i.e. bioobjects (Martinelli et al*.,* 2020). The bio-objects can also be called Genetically Modified Organisms (GMOs). Now, if the target organism is plant, then the resultant modified plant is called as genetically modified (GM)/transgenic plant. Bioobjectification can be done for various purposes viz., higher production, increased nutritive value, disease-insects resistance or tolerance etc. All over the world, first transgenic plant (tobacco) was commercialized in 1996 whereas, in India, genetically modified plant i.e. Bt cotton was commercialized in 2002. India has approved GM crops mainly for three different purposes i.e. food, feed and cultivation. But since last decade, some insect pests viz., diamond back moth *Plutella xylostella* (L.) and pink boll worm *Pectinophora gossypiella* (Saunders) have developed resistance to Bt transgenic crops in all over the world. That is why there is now need to develop a novel strategy for the management of insect-pests that would be more effective and safer for non-target organisms. Now scientists are using bioobjectification process for developing genetically modified insect pests to reduce their natural population. In insects, bio-objectification can be done through two methods viz., physical method and genetic method.

A. Physical methods

1. Sterile Insect Technique (SIT)

In 1930s and 1940s, the concept of SIT was envisaged by geneticist A S Serebrowskii in Moscow; tsetse field researcher F L Van der Planck in Tanzania; and E F Knipling at the United States Department of Agriculture (USDA). The technique includes mass rearing of target insects followed by sex separation as

only male insects are exposed to radiation treatment to make them sterile. These irradiated sterile male insects are released in open field which compete with wild males in mating with wild females and the population of wild females mated by sterile males will lay sterile eggs leading to decline in population. The protocol was commercially first time implemented on cattle screw worm fly (*Cochliomyia hominivorax* Coquerel) and was successfully eradicated from USA, Mexico, Belize, Guatemala, Honduras, El Salvador, Nicaragua, Costa Rica, Panama and Caribbean Islands (Hendrichs et al., 2002). The propitious results of SIT in cattle screw worm fly, International Atomic Energy Agency (IAEA) in 1990, made Anthony Shelton to work on a highly damaging worldwide agricultural insect-pest of crucifers, diamond back moth (*P. xylostella*) and trials were initiated in Indonesia and Malaysia. Though moths were sterilized successfully by radiation, due to some limitations, the project was dropped (Wilke and Marrelli, 2012). The constraints were absence of efficient sex sorting methods, release of both sexes proved inefficient and decrease performance of flying and mating capacity (Alphey and Bonsall, 2018). To overcome these limitations, a more advanced technology of SIT, called inherited sterility came in existence.

2. Inherited sterility (IS)

When a species (mostly lepidopteran) was treated with substerilizing (lower) doses of ionizing radiation, it became partly sterile, but their F1 offspring exhibited a higher level of sterility than the irradiated parents is called inherited sterility or F1 sterility. In general, female insects are more sensitive than males in lepidoptera, hence the method can easily be used for production of irradiated males for open field release. For each specie, radiation dose needs to be standardized at a level that can partially sterilize females but have very limited effect on males. The sub-sterilizing doses of radiation increased the performance of irradiated males in terms of quality and competitiveness. Inherited sterility was first time reported in silkworm, *Bombyx mori* L.; wax moth, *Galleria mellonella* L. and codling moth, *Cydia pomonella* L. and then it was utilized to manage many other insect-pests in different regions of the world (Marec and Vreysen, 2019).

B. Genetic methods

1. Release of insects carrying a dominant lethal gene (RIDL) technology

This technology was first developed by Thomas et al. (2000) from Oxitec Biotechnology Company which is a spin off from Oxford University. It includes introgression of two desired genes viz., lethal gene, tTAV (tetracycline repressible transcription activator variant) and fluorescent marker gene, DsRed2 (a mutant form of DsRed isolated from *Discosoma* sp.). Here, tTAV gene is the dominant lethal gene whereas, DsRed2 is the fluorescent marker gene. These two genes were inserted into eggs of targeted insect with the help of biotechnological tools i.e. micro injection. Once the genes were inserted, the mating between transgenic (genetically modified) male and female produced more numbers of transgenic insects generation after generation. The tTAV lethal gene was inserted into female specific region of genome that would be under control of a female specific promoter. Thereby, the lethal gene would be activated in female insects only; not in male insects rather they acted as carrier.

2. Mechanism of RIDL

Once tTAV gene gets activated in immature stage of a transgenic female insect, it produced tTAV protein which binds with its own control regions in order to initiate a positive feedback loop which means, it would trigger a continuous over production of tTAV protein. Since, all the raw materials (amino acids) had been utilized for the production of tTAV protein; other genes did not produce essential proteins which were mandatory for the survival of insect. Thereby, it proved lethal to insect and thus, the target insect found dead eventually due to indirect effect of tTAV gene (Alphey, 2002). In an interview with Waltz (2015), Neil Morrison, a research leader for agricultural pest control at Oxitec said "All insect-pests reproducing sexually can be managed through RIDL technology". Thus, various insect-pests reproducing sexually were brought under RIDL in order to manage them (Table 1) (Anonymous, 2021).

3. How does RIDL overcome SIT and IS?

The dominant lethal gene, tTAV is also tetracycline repressible hence, its lethal effect can also be suppressed by feeding tetracycline or its analogues to transgenic female insect. So, whenever large scale production of transgenic insects is required, tetracycline or its analogues are added in the artificial diet of rearing insects. This process successfully inherits both the transgenic genes into further generations without reduction of their frequencies. When enough population of transgenic insects are achieved and sex separation is required for the release of only male transgenic

Table 1. Insect-pests covered under RIDL technology

Target insect-pest	Disease/ insect-pest	Details of tansgenic insect-pest	Area of experiment/ progress
Spodoptera frugiperda	Pest of maize in North and South America, Africa and Asia	Friendly™ Fall armyworm	Oxitec and Bayer collaboratively testing in Europe, Brazil and the USA
Soybean looper	Pest of soybean in Asia, America and Africa	Friendly™ Soybean looper	Development is in early stage
Mediterranean fruit fly	Pest in Africa, the Mediterranean, South and Central America, parts of Australia	Friendly™ Medfly	Oxitec and DPIRD (Department of Primary Industries and Regional Development) tested performance in laboratory successfully and waiting for Australian Government for open field trials
Spotted wing drosophila	Pest in Eastern Asia, North America and Europe	Not yet developed	Development of Friendly™ Spotted wing drosophila is under progress
Diamondback moth	Pest of crucifers in all over the world	Friendly™ Diamondback moth	Oxitec and Shelton lab (Cornell Uni.) conducted laboratory, field cage and open field cage trials and achieved positive results
Aedes aegypti	Vector of dengue, Zika, chikungunya and yellow fever	Friendly™ *Aedes aegypti*	Pilot project in Indaiatuba, Brazil suppressed wild population successfully
Anopheles albimanus	Vector of malaria in meso America	Friendly™ *Anopheles albimanus*	Development is at an early stage
Anopheles stephensi	Vector of malaria in Asia	Friendly™ *Anopheles stephensi*	Development is at an early stage

insects, tetracycline or its analogues are not added to the artificial diet of transgenic insects. This would kill all the female transgenic insects while, transgenic males would survive as normal. That is why it is called male selecting (MS) and self-limiting genetic system (Heinrich, 2000; Thomas, 2000; Wyss, 2000). The fluorescent marker gene produces DsRed2 protein in all the body parts of the insect during all life stages viz., larva, pupa and adult; except egg. This protein makes immature stage of insect glow under specific fluorescent light by which transgenic strain and normal-wild strain of insect can easily be identified visually. The DsRed2 protein is nontoxic and non-allergenic (Wilke and Marrelli, 2012). These features make RIDL overcome SIT and IS.

a. Mosquito *Aedes aegypti* L.

By introducing tTAV and DsRed2 genes into dengue mosquito, *Aedes aegypti* L., a transgenic strain OX513A was developed. Open field release of OX513A males was carried out in Cayman Islands (UK) (2009-10), Malaysia (2010-11) and Brazil (2011-12) (Beech et al., 2013). In Cayman Islands, up to 96% wild mosquito suppression was achieved with help of OX513A strain (Wilke and Marrelli, 2012). In India, effectiveness of *A. aegypti* OX513A strain was assessed against Indian strains, collected from New Delhi and Aurangabad. For that, Oxitec collaborated with Gangabishan Bhikulal Investment and Trading Limited (GBIT) and conducted laboratory trials in Arthropod Containment Level - laboratory in 2011 and field cage trial in 2017 at Dawalwadi, Maharashtra. Performance of OX513A was compared with Indian wild strains in case of mating, progeny sired, reproductive and growth parameters. In all these parameters, OX513A performed similarly with wild mosquitos collected from New Delhi and Aurangabad (Patil et al*.,* 2015). Males and females of all strains showed cent per cent mortality in case of malathion 5%, deltamethrin 0.05% and permethrin 0.75%. In case of DDT 4%, male OX513A showed the highest mortality (90.9%) after 24 hours of application with susceptibility status of "resistance likely" whereas, female OX513A also showed the highest mortality (70.1%) but with susceptibility status of "resistance" category (Patil et al*.,* 2018).

b. Diamond back moth *P. xylostella*

Diamond back moth transgenic strain (OX4319L) was also developed by inserting tTAV and DsRed2 genes in the wild diamondback moth strain named Vero Beach. Response of transgenic diamondback moth (OX4319L) was compared and evaluated with other wild strains of diamondback moth (Vero Beach, Geneva 88 and Georgia) towards synthetic pheromone (ISCAlure-Xylostella) which was placed at the top of a vertical wind tunnel (2m height with air speed of 0.5m/s flow from bottom to top). The moths were placed at the bottom and the behaviour was found similar among all four strains of diamondback moth on the basis of three check points whether they take flight, fly upwind direction or reach close to lure. This result confirmed that, transgenic and wild diamondback moth strains showed almost similar response towards female sex pheromone (Bolton et al*.,* 2019).

Further, during field cage experiment in 2015, OX4319L strain successfully crashed wild diamondback moth population with improved longevity and mating performance than irradiated diamondback moth population. Collaboration of Oxitec Ltd. and Cornell University in 2017-18, the world's first open field release of a self-limiting and genetically engineered agricultural insect-pest (diamondback moth) was carried out at Cornell University's New York State Agricultural Experiment Station (NYSAS) in Geneva, New York. Shelton et al. (2019) carried out open field experiment to evaluate the performance of OX4319L strain of diamondback moth in cabbage cv. cabton grown circularly in 2.83ha area with 10m of surrounding buffer bare area wherein OX4319L and wild strain of diamondback moths were released at the centre of field on different dates with varying release rates i.e. 1000, 1500 and 2500 moths and each release rate moths were marked with a different coloured fluorescent powder to identify when they recaptured. To recapture them, 48 sticky pheromone traps were also placed at 7m, 14m, 21m, 28m, 35m, 55m, 75m and 95m distance from the centre of field in circular pattern and each trap was collected and replaced daily after each release until no marked diamondback moth was detected in any trap for two consecutive days.

The result of the trap catches indicated that male moths of OX4319L strain effectively suppressed the population of wild diamondback moth. In case of persistence in field, transgenic and wild strains did not show any significant difference. Shelton et al. (2019) found that OX4319L strain showed relatively higher per cent survival than wild strain of diamondback moth. Based on overall per cent recovered moths in traps, OX4319L strain showed significantly lower (7.5%) per cent recovery than that of wild strain of diamondback moth. But overall total proportion recaptured in the first 7-35m for wild strain (97.8%) and OX4319L (95.4%) were almost similar which was encouraging for OX4319L diamondback moth. The mean distance travelled by moths indirectly reflected their flying capacity in open field in which OX4319L strain travelled significantly higher (50. 2m) distance than wild moths (29.9 m).

c. Pink boll worm *P. gossypiella*

A strain of pink bollworm, OX1124 was developed by micro injecting tetO (tetracycline operator element) gene expressing high production of tTAV protein and ZsGreen gene (fluorescent marker). Further, four lines were developed viz., OX1124A, OX1124C, OX1124D and OX1124E in which, OX1124A and OX1124D lines showed the highest (87-96%) survival of pink bollworm to adult stage in the presence of chlortetracycline and the lowest (27-33%) survival in the absence of chlortetracycline. It clearly meant that OX1124A and OX1124D were the most competent and were appropriate for field evaluation. Scientists theorised that by increasing copy number of tetO cassette would proportionately amplify lethality in pink bollworm. In order to test this hypothesis, a crossed line OX1124AD (OX1124A and OX1124D) was developed which exhibited the least (1.9%) survival to pupae. This experiment proved that increasing copy numbers of tetO gene cassette also increases mortality in pink bollworm. With a view to above experiment, scientists developed two lines, OX3347A and OX3400A from OX3347 (tTAV2:tTAV3 = 21:7 tetO repeats) and OX3400 (tTAV2:tTAV3 = 21 tetO repeats with inverse orientation) strains, respectively. Both lines showed survival per centage very high with chlortetracycline application and low without chlortetracycline application. But this lethality was recessive and the lines were homozygous in nature which would not prove efficient in open field.

Six new transgenic lines, OX3402A, OX3402C, OX3402M, OX3402P, OX3402T and OX3402U were developed from OX3402 strain having tTAV2 and tTAV3 with tandem orientation. Here, instead of ZsGreen, DsRed2 fluorescent marker gene was utilised. In tests, OX3402C and OX3402P exhibited appreciable results but OX3402P was composed of

recessive lethal mutation. Hence, OX3402C was further selected for its field efficiency. But mortality in pink bollworm must occur during its early instar life stage; otherwise, late life stage mortality would definitely result in suppression of population of pink bollworm but only after cotton bolls had been damaged. For that reason, damage by pink bollworms on cotton bolls was evaluated in caged field condition and compared with wild, OX3402C heterozygous and OX3402C homozygous pink bollworm releases. The cage with wild pink bollworm release showed adults emergence from pupae as well as larvae also damaged to bolls and lint. The cage with release either of OX3402C heterozygous and homozygous showed no any adults emergence. However, at some extent, entry holes and minor lint damage were present but most of larvae found dead in their early instar life stage while, some were found stunted and inactive (Morrison et al., 2012).

d. Resistance management in insect-pests through RIDL

In order to determine whether transgenic insects can be used to reverse the resistance to any insecticide or to Bt in wild insect population or to delay in resistance development. A field cage experiment was conducted by Samuel et al. (2015) who evaluated high and low release rates of OX4319L along with various combinations of Bt and non Bt broccoli having wild-resistant diamondback moths. After passing of four generations of diamondback moth in the cage experiment, they found significantly lower population of wild-resistant diamondback moths in cage wherein OX4319L were released at low rate along with Bt broccoli and wild-resistant diamondback moths (Fig. 1). The data confirmed that release of OX4319L strain can diminish the population of wild-resistant diamondback moth on Bt broccoli and it can also be utilised for reducing resistant population.

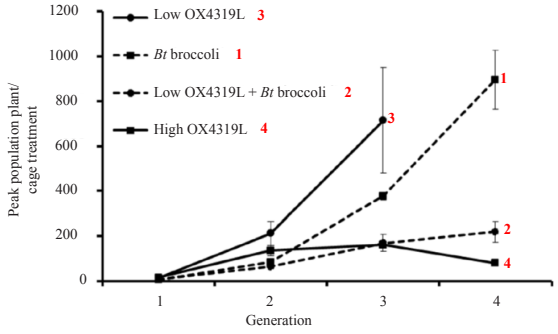

Fig. 1. Effect of Bt broccoli and OX4319L releases on caged *P. xylostella*

Samuel et al. (2015) conducted another experiment retrieving population of diamondback moth resulting from earlier experiment to determine how much proportion of survived populations of diamondback moth can tolerate high level of Bt artificial diet. In findings, they recorded significantly lower (39.5%) survival per cent in the population resulted from the cage containing Bt broccoli+low rate weekly OX4319L releases than only Bt broccoli cage population (89.7%) (Fig. 2). This confirmed that OX4319L strain can not only reduce the Bt resistant diamondback moth population but also reduce or even delay the resistance developed in wild diamondback moth population. The results could also be confirmed on the basis of fluorescent moth population present in remaining survived total diamondback moths. Cages containing Bt broccoli+low rate weekly OX4319L releases and non Bt broccoli+low rate weekly OX4319L releases were observed with almost similar fluorescent proportion of diamondback moth (56% and 55.6%, respectively). The fluorescent proportion showed the proportion of wild-resistant diamondback moth which had been transferred with lethal gene by OX4319L strain. The findings confirmed that wild-resistant population of insects can also be converted into susceptible population with help of RIDL and easily be managed.

CONCLUSIONS

Bioobjectified/ genetically engineered insects target only their own species. They do not harm beneficial insects. There is no use of chemicals that would be harmful to environment and ecology. The self-limiting gene cannot establish in the ecosystem permanently. The tTAV protein does not produce any toxins or allergens. With help of RIDL, the production of genetically engineered insects at large scale became very easy. These self-limiting insects can be used as part of integrated pest management or even as a stand-alone solution (Anonymous, 2021).

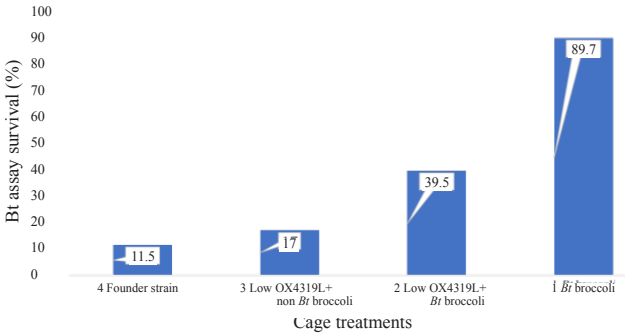

Fig. 2. Survival of *P. xylostella* fed with Bt artificial diet

Bioobjectification using release of insects carrying a dominant lethal gene (RIDL) system is a novel approach for insect-pest management. Use of RIDL with transgenic mosquito (OX513A) very efficiently suppressed wild mosquito population. Besides mosquito, transgenic strains of diamondback moth, OX4319L and pink bollworm, OX3402C have been developed which showed excellent suppression of wild insect population. Males of transgenic diamondback moth strain (OX4319L) containing Bt susceptible background can manage or even reverse the resistance developed in wild-resistant diamondback moth population. Male selecting insects carrying Bt susceptible genetic background helped to reduce or partially replace the refuge requirements as a resistance management tool in Bt crops. Thus, bio-objectification has created a wider opportunity for futuristic insect-pest management. Though there is still need to appraise this technique against major insect-pests of crops that have already developed resistance to many insecticides. Although there is still need to evaluate and successfully incorporate this technology as a component of integrated pest management system.

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