ROLE OF BT. GENE IN CONTROL OF INSECT PESTWITH SPECIAL REFERENCE TO DIAMONDBACK MOTHS

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ABSTRACT

These studies have documented that Bt-resistant insects can survive on Bt plants and that different management strategies will influence the durability of resistance. Although these studies provided some insight into variables that could be manipulated to delay the onset of resistance, the present field study was performed to provide further data to help identify variables that may influence resistance management in the field. Field experiment examined the effect of refuge size and refuge placement (mixed vs. separate refuges) on the distribution of the larvae within the plots as well as the level of resistance in diamondback moths at the end of the season. Our results demonstrated that the cumulative number of larvae per plant on refuge plants through the season in the 20% mixed refuge was significantly lower (6.4 vs. 14.6) than the 20% separate refuge (Table 1). This finding indicates that, as in our previous greenhouse experiments, a separate refuge is more effective at conserving the number of susceptible alleles because larvae on these refuge plants will be more likely to survive to adults (either SS or RS) that can mate with RR individuals and thereby reduce the number of RR offspring. This finding provides evidence to support the use of a separate refuge for Bt-transgenic crops that are attacked by insects that can move between plants as larvae. On the Bt-expressing plants over the season, an average of £0.3 larva was found in any of the treatments, indicating that the diamondback moth population was being controlled by the Btexpressing plants (Table 1). This was also confirmed by the absence of any larvae on the Bt-expressing plants at the end of the season

Keywords: *Durability of resistance, refuge, greenhouse experiments, treatments.*

INTRODUCTION

Traditional breeding has done much to improve the host plant resistance of crops to insect pests and continuous to provide new varieties of crops which require less chemical intervention than old varieties. Such as glabrousness (absence of hairs on leaves), frego bract (outward bending, thin bracts around the crops balls), nectar less (absence of nectar glands on the leaves and flowers) and high gland contents are now being assessed for their capacities to reduce the attractiveness of the crops plants to insect pests. There is however limit to the improvement in natural resistance to insect provided by alterations in plant shape and structure. Up to now none ofthe crops varieties developed so far has shown more than a moderate level of resistance.

Therefore, control on insect pest in crops varieties developed so far has shown more than a moderate level of resistance. Therefore control of insect pests in crops cultivation depends mainly on the use of insecticides that are under serious public debate for reasons of human safety and environmental pollution. Scientists have been looking for new strategies to control insect pests. An alternative is the production of proteins with insecticidal activities by the crops plant itself. Genetic engineering should enhance the capacity to produce the more tolerant plants by accessing a much wider gene pool for novel insect resistance characters not present in any of the Gossypium species or their close relatives. Numerous laboratories and field tests confirm that the most effective and cheapest method for protection crops from pest is the utilization of transgenic crops for insect resistance.

REVIEW OF LITERATURE

The most widely favoured genes though to be most useful for crops is the Bt toxin gene which contain a crystalline protein toxin. Bt. Toxins are insecticidal proteins found as parasporal crystalline inclusions in sporulated Bt strains. They are characterized by their potency and their specificity towards specific insect pest, many of which are agronomically important and their relative safety to non-target insect species and vertebrates, particularly human being. They have been used to control crops pests for more than 30 years in USA. Similar Bt genes are identified and inoculated in tobacco, tomato and a number of other crop plants where they have increased tolerance to insect pests.

China started developing transgenic Bt. Crops in the late 1980s and the first crops plant was developed in 1991.

Fan and coworkers obtained Bt. Crops carrying cry1a (b) and cry1(c) genes from Bt species kurstaki strains HD-1 and HD-73 respectively.

The pink ball worm (Pectinophora gossypiella) was described first in 1843 by W.W.Saunders as Depressaria gossypiella, from specimens found to9 be damaging crops in India in1842. This insect pest is distributed all over the world. It is very common in U.P., M.P., Punjab, Haryana, Tamil Nadu and Maharashtra. It draw nutrients from inside of the crops seeds and cause serious loss to the crop (W.W.Saunders 1843)

Landers E.S., 1996, explain the plant genomics as to revolutionizing our understanding of biology as never before. It deals with the study of whole genomes of plant which helps in DNA chip technology.

Smith and Smith L.M., 1986 studied the laser excitation of bands of newly synthesized DNA, sensing of colours by photomultipliers and finally automatic output of sequence data through computers attached to sequencers.

Cooper, N.G., 1993, detected the spots of hybridized DNA by autoradiography and corresponding clone on the microtitre plate

Olson, M.V. Hood, 1989, gave the idea of overlapping of genes in a clone hence the concept of marking the genes.

Kulson, A.M. and Water J., 1989, suggested probes for identifying the new YAC clones to bridge the gaps. However, in certain cases earlier cosmid based contigs were constricted a large YAC clone is used to bridge the gap.

Skata. K. 2000, Studied on a new database INE(Integrated Rice Genome Explorar) now established as I-ne. the rice genome is estimated to comprise -430 Mb DNA, which is the four time the amount of DNA in Arabidopsis.

Richmon D,T. , 2000, Studied the gene probes of various plants through the use of DNA chip. Asto determine the sequence of genes in rice and other crop plants.

A lot of work has been carried on by different workers in last two decades still it is the initial stage of Biotechnology which have vide scope in future research.

MATERIAL AND METHOD

Cotton crop was taken as the study material as it is one of the cash crop in Haryana and a large area is under cultivation in district Mahendergargh, Bhiwani and Rewai Hisar and Sirsa. The cytological studies on Bt. Crops and other varieties of cotton crop were taken under consideration in the laboratory.

The identification of other genes for insect resistance such as those for proteinase inhibitors, alpha amylase inhibitors and lectins was done as the effective genes even for other crop plants as attacked by insect pests. The effectiveness and mode of action of products of these genes was studied as to know the physiology of insect after consumption of such plant products which are modified through these genes. Bio-assay tests were carried on as to determine the effectiveness of the gene. Help from Institute of Immunology, Delhi University, and Guru Jambheswar Technical University, Hisar (Haryana) was taken where research work of the Guide is already in progress. Other types of effective genes were identified on the basis of their effectiveness and suitability for a particular crop.

These studies have documented that Bt-resistant insects can survive on Bt plants and that different management strategies will influence the durability of resistance. Although these studies provided some insight into variables that could be manipulated to delay the onset of resistance, the present field study was performed to provide further data to help identify variables that may influence resistance management in the field. Field experiment examined the effect of refuge size and refuge placement (mixed vs. separate refuges) on the distribution of the larvae within the plots as well as the level of resistance in diamondback moths at the end of the season. Our results demonstrated that the cumulative number of larvae per plant on refuge plants through the season in the 20% mixed refuge was significantly lower (6.4 vs. 14.6) than the 20% separate refuge (Table 1). This finding indicates that, as in our previous greenhouse experiments, a

separate refuge is more effective at conserving the number of susceptible alleles because larvae on these refuge plants will be more likely to survive to adults (either SS or RS) that can mate with RR individuals and thereby reduce the number of RR offspring. This finding provides evidence to support the use of a separate refuge for Bt-transgenic crops that are attacked by insects that can move between plants as larvae. On the Bt-expressing plants over the season, an average of £0.3 larva was found in any of the treatments, indicating that the diamondback moth population was being controlled by the Btexpressing plants (Table 1). This was also confirmed by the absence of any larvae on the Bt-expressing plants at the end of the season. In leaf-dip assays taken through the season, no differences in susceptibility were detected between diamondback moths taken from any of the treatments (Table 2). Furthermore, comparing the level of resistance at the beginning of the test to the level at the end, it appears that the insects actually became more susceptible. This was the result of immigration of native susceptible diamondback moths into our field plots, which diluted the frequency of resistant alleles of the released insects and prevented the establishment of resistance even when R allele frequencies of released larvae were as high as 0.12. This result was not seen in our previous greenhouse studies in which we had a closed system prohibiting immigration. Despite the differences in the number of larvae on refuge plants in the mixed and separate refuges in this field study (Table 1), we were not able to document differences in mortality (Table 2) over the relatively short period of this experiment. However, the differences in larval populations on the refuge plants in these treatments do lay the groundwork for differences in susceptibility to occur given a longer time period. Our results from this field study might be taken as justification for not needing any refuge within a planting because of the presence of immigrating susceptible alleles. However, such an approach would only be justified if immigration patterns of susceptible insects were well known and had been shown to be consistent. Usually one does not know a priori whether such immigration of susceptible alleles will occur. Under conditions in which there is no such immigration, high levels of resistance and crop damage can occur16.

Growers may be unwilling to sacrifice large numbers of refuge plants to delay the onset of resistance. Thus, current recommendations allow the management of insects on these refuge plants through the use of insecticides with a different mode of action than the Bt-transgenic plants. The critical question in such a strategy is Number of larvae per plant whether enough susceptible insects will survive in the refuge to provide an effective source of susceptible alleles. Because there is no documented cross-resistance between Cry1C and Cry 1A BT toxins18, 19, we examined how spraying the refuge with M-C (Mycogen, encapsulated Cry1C) affected DBM larval density and resistance on Cry1Ac broccoli. Our results indicate that in both 100% refuge treatments (where insects were released or where insects were not released), susceptibility increased significantly over time (Fig. 1). With a discriminating dose of 10 p.p.m., the population had a rate of 27% mortality before release into the treatments, but in both 100% refuge treatments the mortality at 10 p.p.m. increased to >70% by the third count. The similar increase in susceptibility in both treatments is indicative of immigration of susceptible insects into those plots, as was also seen in the 1996 field studies. However, despite high rates of immigration of susceptible insects, when resistance allele frequencies in the plot were high, spraying the refuge resulted in progressively higher levels of resistance over the course of the season than when the

refuge was not sprayed (Fig. 1). In both the second and third counts, the insect population in the sprayed refuge had a significant and >15% lower average mortality at the diagnostic dose for resistance (10 p.p.m.), compared with the insects in the unsprayed refuge. Insects collected from the Bt plants would have a RR genotype for Bt var. kurstaki resistance, and we consistently found significantly higher numbers of Bt var. kurstaki-resistant larvae on the Bt plants when the refuge was sprayed than when it was not sprayed (Fig. 2). This is the opposite of what should occur if resistant alleles are to be maintained in the refuge for an effective resistance management strategy.

To illustrate this further, we examined the overall diamondback moth population within our experimental plots of 300 broccoli plants. Because each 20% refuge plot had 240 Bt plants and 60 refuge plants, a higher number of larvae per Bt plant translated to a significantly higher overall population in the plot in the second and third counts when the refuge was sprayed than when not sprayed (Fig. 3). The important point demonstrated here is that spraying the refuge reduces its potential to dilute resistance. By leaving the refuge unsprayed and giving more susceptible insects a chance to survive, short-term sacrifices of relatively more insects in the refuge may translate to seasonal reductions in resistance and reductions in the total number of larvae per plot. The critical question is whether such populations would result in unacceptable crop losses. The high-dose/refuge strategy is the current foundation for managing pest resistance to Bt plants. Whereas the consensus is that the efficiency of this strategy depends on early implementation before the frequency of resistance alleles is high, evaluation under field conditions with this criterion is inherently difficult. We can approach such an evaluation by increasing the R allele frequency, as we did with multiple releases, and then assess changes in susceptibility and effectiveness of the refuge in conserving susceptible alleles within a field. Our results indicate that the use of refuges can be a sound strategy. However, this strategy will also depend on our ability to effectively monitor and manage susceptible alleles on an individual field or farm basis as well as on an areawide basis. Within an individual field or farm, treating the refuge with a highly effective insecticide may dilute the abundance of susceptible alleles to such an extent that the refuge is rendered ineffective unless there is substantial immigration of susceptible alleles from wild hosts or from surrounding non-Bt crops. On the other hand, growers may be reluctant to sacrifice a large number of refuge plants to insects just to maintain susceptible alleles. An alternative to the strategy of having a 20% refuge that can be sprayed (the requirement for crops) is the EPA-approved strategy (also in crops) of having a 4% refuge that remains unsprayed. Critical experiments need to be performed to assess which approach, as well as which refuge size, would be more effective in conserving susceptible alleles while providing acceptable crop yields, and such tests need to be performed in the specific insect/Bt crop system.

CONCLUSION

As we refine resistance management strategies for the currently available Bt crops, it is also imperative that other strategies for managing overall resistance to Bt be developed and implemented in the near future. Having Bt expressed in plants so that the insect population is subjected to selection pressure for particular periods of time.

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