MEETING

ABSTRACTS OF LECTURES ON MOLECULAR BIOLOGY TO ADVANCE PLANT PROTECTION IN THE 2000s PRESENTED AT

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Activities of the Israeli National Committee for Transgenic Plants

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The Ministry of Agriculture's Chief Scientist appointed the National Committee for Transgenic Plants (NCTP) in 1991. Its mandate is to regulate research and breeding programs utilizing transgenic plants and techniques. The 12 members of the committee represent the academic community, the public and the government (the ministries of Agriculture, Environment, Science, Health, and the Prime Minister's Office). The main roles of the NCTP are: (i) to formulate guidelines for research with transgenic plants, including organisms interacting with plants such as plant pathogenic nematodes, symbiotic or pathogenic fungi and bacteria, insects, viruses and viroids; (ii) publication of protocols and application forms for genetic engineering experiments with plants; and (iii) advising government and research institutions as to good practice concerning importation and experimentation with transgenic plants.

A panel of experts in the fields of molecular biology, environment, and biology (genetics, botany and entomology) reviews applications for experiments with transgenic plants, according to the subject submitted. Applications for field experiments are reviewed in plenary sessions. Teams of Plant Protection and Inspection Services (PPIS) inspectors and NCTP representatives inspect field and glasshouse experiments. The experiments carried out in Israel are in the areas of yield improvement (parthenocarpy and shelf life), resistance to diseases, pests, herbicides, and various stress agents (drought, salinity, etc.).

Currently, the NCTP deals with about 100 registered projects, approximately 10% of which are field experiments. Most of the projects are carried out at universities and research institutes, with a small proportion being conducted by seed companies and nurseries, usually with imported transgenic material and in cooperation with foreign companies or institutions. The crops and plants now under investigation are: tobacco, potato, tomato, lemon, zucchini, strawberry, wheat, alfalfa, vine, *Arabidopsis,* petunia, ginseng, poplar, carnation, pine, and microorganisms – the last mentioned mostly for biological control.

The NCTP is presently preparing the transition from protocols to legislation.

Developing Molecular Technologies for Crop Protection

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The last decade was characterized by intensive development of technologies for plant protection using molecular techniques. Plant transformation with foreign DNA became a tool which is applicable to many crops, both mono- and dicots, using mostly *Agrobacterium* and micro-projectile bombardment for DNA delivery. Many useful genes and promoters have been isolated and utilized in order to achieve general, induced, or tissue-specific expression of the transgene. Transgenic plants harboring foreign DNA were produced using genes isolated from different plant species or other organisms, such as viruses, bacteria, fungi and animals. The level of expression of many genes has been manipulated in transgenic plants by their over-expression utilizing different promoters, or suppression by antisense RNA production, co-suppression and gene-specific ribozyme utilization. Resistance genes for several pathogens have been isolated, including resistance to viruses, bacteria, fungi and nematodes and some were used already to transfer the resistance to different crops. These genes share similar structural motifs, suggesting that they serve as components of signal transduction pathways. Transgenic plants have been produced which are characterized by resistance to different pathogens and pests, examples of which are: insect resistance induced by the BT gene, virus resistance by antisense or coat protein production, fungal resistance by expression of fungal cell wall hydrolases, ribosome-inactivating proteins, cystein-rich antifungal peptides, different PR proteins, phytoalexins, etc. Plants resistant to most herbicides had also been obtained including resistance to Roundup (glyphosate), Basta (glufosinate), 2,4-D, dalapon and bromoxynil. As more is being learned about the molecular biology of resistance mechanisms, more sophisticated schemes for production of resistant plants are employed, and the promise of using transgenic plants for crop protection is becoming a reality.

Impact of BT-transgenic Cotton on the Main Israeli Lepidopteran Cotton Pests

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Insecticides produced by fermentation of *Bacillus thuringiensis* (BT) have been used since the 1930s. These insecticides are considered safe and environmentally friendly; they are lethal to certain lepidopteran, coleopteran and dipteran insects (depending on the specific toxin); and cause no harm to other organisms including mammals, wildlife, natural enemies, and other non-target species. Unfortunately, these products are nonpersistent outdoors, and since BT toxins act in the insect midgut, they must be ingested to be effective. For this reason, the effect of BT-spray on bollworms and other pests that penetrate into fruits is limited.

Through genetic engineering, transgenic crops like cotton, corn and potatoes express BT toxins. Transgenic cotton plants, introduced commercially into the USA and Australia in 1995-96, were found to be protected from pests such as the *Heliothis (Helicoverpa)* complex and the pink bollworm, *Pectinophora gossypiella.* One of the main problems with the use of transgenic crops is the potential evolution of resistance, because of the continuous expression of the BT toxins. Extensive use of BT-transgenic crops might cause loss of the effectiveness of both transgenic crops and BT-spray products. To prevent or delay the onset of resistance in transgenic crops, it has been suggested to use plants expressing high doses of the toxins and to establish large areas of refuge plants near fields of transgenic cotton.

Following the short experience of commercial use of transgenic cotton worldwide, it was found that the number of insecticide applications could be reduced, but the crop was not protected uniformly from attack by various pests, even within the same genus. In addition, the expression of toxins sometimes declined during the cotton season and varied with the cropping conditions.

In Israel the main lepidopteran cotton pests are: the pink bollworm, *P. gossypiella;* the spiny bollworm, *Earias insulana;* the cotton bollworm, *Helicoverpa armigera;* the Egyptian leafworm, *Spodoptera littoralis;* and, occasionally, the looper *Chrysodeixis chalcites.* Since 1987, an insecticide resistance management (IRM) strategy has been implemented in cotton in Israel. The main goal is to delay the onset of resistance by using insecticides in a rational way. This strategy is considered successful worldwide; however, the main lepidopteran pests, the pink and the spiny bollworms, can be controlled only by successive nonselective insecticide applications. BT-transgenic cotton in Israel would be protected against pink, spiny and cotton bollworms as well as against the looper *C. chalcites.*

Pathogen-Derived Resistance to Viruses

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The concept of pathogen-derived resistance (PDR) against viruses has evolved from viral cross-protection. In cross-protection, a mild or attenuated strain of a virus is used to protect crops from infection by a virulent strain of a related virus. In 1985, Sanford and Johnston proposed that the expression of viral genes in transgenic plants may interfere with the proliferation of an incoming virus and thus confer resistance. The first demonstration of PDR was reported in 1986, when transgenic tobacco plants expressing the capsid protein (CP) of tobacco mosaic virus (TMV) were shown to express a high level of resistance to TMV infection.

Taking TMV as a model system, all the viral open reading frames have been shown to induce resistance once expressed in transgenic plants. Expression of TMV CP in transgenic plants induced a high level of resistance against TMV and related viruses. Replicase (54K) mediated resistance induced a very high level of resistance, practically immunity, but the resistance was strain-specific – effective only against the virus the transgene was taken from, *i.e.*, TMV. However, when a modified replicase was expressed in transgenic plants, a high level of broad-spectrum resistance was obtained, being effective against all tobamoviruses tested. Another approach was the expression of a mutated dysfunctional movement protein (dMP) in transgenic plants; dMP mediated resistance was very broad-spectrum – effective against viruses from seven different taxonomic groups, but the resistance level was not high.

Undoubtedly, breeding plants for resistance is the best approach to inhibiting virus spread. However, the identification and introduction of new resistance genes to crop plants is a challenge. Most new resistance genes are identified in wild species and their incorporation into crop plants is problematic, primarily due to sexual incompatibility barriers, and the introduction of undesirable traits alongside the resistance which necessitates extensive back-crossing. In contrast, PDR offers the ability to introduce resistance genes into crop plants regardless of their sexual compatibility and without compromising existing desirable agronomic traits. Combining conventional breeding with PDR offers the introduction of a large number of new resistance genes with the ability to create different multiple resistant gene combinations.

Herbicide-Resistant Crops: Risks and Benefits

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The recent commercialization of crop cultivars containing transgenes for herbicide resistance (HRC) in North America, Europe and other parts of the world, constitutes a potential blessing to agriculture but poses as well a potential threat to the well-being of the agro-ecosystem. Hundreds of transgenic plants were released in 1997 in EU countries and thousands in the USA. The introduction of HRC into the farming system may facilitate the use of environmentally friendly herbicides and improve the cost-effectiveness and profitability of weed control. It may result in reduction of (*a*) the damage caused to crop plants by misuse of herbicides and (*b*) the need for manpower for hand-weeding. However, too hasty adoption of HRC may result in irreversible damage to the agro-ecosystem. The use of HRC will increase the dependence on chemical control methods and may tempt the farmer to use higher rates and repeated applications of herbicides. This is particularly true when the herbicide is generic and cheap. Above all, the selection pressure on the weed population will increase and that will shift it toward higher infestation with troublesome weeds and eventually may facilitate the evolution of herbicide-resistant weeds. Furthermore, the cultivation of transgenic crops with herbicide resistance traits such as oil seed rape, wheat, sugar beet and rice may result in gene transfer by cross-pollination with wild-relatives weeds, resulting in the formation of 'super weeds'. It will also increase the problem of volunteers infesting the crops following in the rotation. Strict risk assessment and careful measures should be taken before any introduction of HRC into the Israeli farming system.