### BIOTECHNOLOGY FOR DEVELOPING HOST-PLANT RESISTANCE TO BIOTIC STRESSES

Wojciech K. Kaniewski, Roger N. Beachy

Abstract. Biotic stresses cause substantial yield and quality loses in all crops, especially in developing countries. It would be easier and less costly to prevent those losses by introducing crops with conventional or transgenic disease and pest resistance, rather than implementing more classical agricultural practices (e.g., use of certain types of agrochemicals) in such countries. Immediate implementation should proceed with insect and virus resistance that have received approval in other countries, with continuing research toward developing strategies to adapt relevant technologies to develop local insect and virus resistant crops. Future programs with resistance to pathogenic fungi, bacteria and nematodes should be implemented as available. The pre-condition for the development of all such programs in developing countries is the adoption of regulatory approval laws as well as local public acceptance.

**Key words:** biotic, transgenic, resistance, weeds, insects, viruses, fungi, bacteria, nematodes

### INTRODUCTION

There are a large number of biotic stresses that can reduce yield and quality in cultivated crops around the world. Losses due to weeds, birds or animals can be minimized by direct farmers care. Insects, nematodes, fungi or bacterial diseases can be effectively controlled using appropriate pesticides, though it is a costly process and often unavailable to subsistence farmers in developing countries.

Modern weed control strategies include herbicide resistant crops developed by genetic engineering [Gressel 2002]. This is a very effective approach, but requires planting of herbicide resistant crop and application of appropriate herbicide. It was fast and easy adapted by moderate and large scale, technically advanced farmers. Small scale farmers in developing countries could highly benefit using this technology by saving time for hand weeding and having increased crop yield. At present herbicide resistance is commercially used in soybean, corn, cotton and canola [James 2003], with potential in many other crops.

Transgenic insect control is currently based on expression of genes derived from various *Bacillus thuringiensis* (Bt) strains [Jenkins 1999]. Bt genes are commercially used to protect cotton, corn, and potato from lepidopteran and coleopteran pests [James 2003]. Furthermore such genes are relevant to many types of farm and crop conditions

and are especially desirable in developing countries. Use of Bt genes usually reduces the requirement for additional inputs, and protecting crops from insects creates substantial economic savings for the farmer. The search for Bt genes and genes from other organisms to protect crops against additional insect pests is currently underway.

Effective control of fungal and bacterial diseases continues to rely on pesticides and classical resistance genes, although research toward transgenic resistance is underway [Honee 1999, Rommens and Kishore 2000]. The most promising approaches include the use of antifungal proteins and mechanisms that employ hypersensitive response (HR) or systemic acquired resistance (SAR). Research toward transgenic nematode resistance is progressing, though products are not expected in the near future [Opperman and Conkling 1998]. When successful, crops that are resistant to fungi, bacteria and nematode will be highly desirable in developing countries.

There is no single preventive measure to protect crops from the many different viruses or viroids that infect crop plants. At present, farmers can minimize losses caused by viruses by using resistant varieties (if available), though such varieties often deliver inferior yield and food quality. Other plant virus control measures consist of seed certification to ensure virus-free material, crop rotation or isolation, removing infected plants, controlling weeds that harbor viruses and insect vectors, and controlling insects using pesticides. The most drastic and often unacceptable virus control measure is called "cross protection". Here, infection of the crop with mild strain of virus might prevent more severe virus strain infection. Cross protection can cause some yield losses and may be harmful for crops that are grown in nearby fields.

Despite all efforts, plant viruses cause average yield loses of \$60 billion a year in major crops [Cann 1997]. Grower expenditures to minimize yield losses through management and pesticide greatly exceed the cost of yield losses.

Modern genetic engineering can introduce virus resistance into cultivated crops [Kaniewski and Lawson 1998]. Such resistance is heritable, durable and does not cause yield reduction. Once introduced, it can be delivered in planting materials and used without additional expenses and efforts.

### SCIENCE AND TECHNOLOGY OF VIRUS RESISTANCE

With the advent of modern biotechnology, virologists searched for genes that would confer resistance to specific viruses. Naturally occurring resistance genes were difficult to locate in the large genomes of higher plants, and the concept of using genes from the pathogen was adapted as an experimental approach. Pathogen-Derived Resistance (PDR) relies in theory on using a gene or part of a gene from a pathogen to interfere directly or indirectly with the development of infection or disease caused by the pathogen. Such genes, when introduced as a transgene to plants, would confer a level of resistance. Several types of virus gene sequences were tested with different levels of success. Coat protein and replicase genes conferred significant levels of resistance to many economically important plant viruses, though other genes also have potential. Complete understanding of mechanisms of different types of pathogen-derived resistance are not yet at hand; mechanisms can vary depending upon the virus and its mode of infection and replication. In spite of not being completely understood, transgenic pathogen derived resistance to plant viruses can be of high practical value.

### TECHNOLOGY APPLICATIONS AND DEVELOPMENT

The concept of PDR was first demonstrated on tobacco and tomato plants to protect against tobamoviruses [Powell et al. 1986, Nelson et al. 1988]. Later, various crops were transformed with different virus-derived genes, where the resistant lines were selected for evaluation under field conditions [Kaniewski and Thomas 1993, Kaniewski and Thomas 1999]. Prior to commercialization, regulatory approval processes needed to be completed.

The first commercialized transgenic virus resistant plant was in China in 1992 and involved tobacco plants that were resistant to tobacco mosaic or cucumber mosaic virus [James and Krattiger 1996]. In the US, commercial approvals were granted for squash (watermelon mosaic virus, cucumber mosaic virus and zucchini yellow mosaic virus), potato (potato virus Y and potato leafroll virus) and papaya (papaya ringspot virus). Tomato resistant to cucumber mosaic virus was successfully generated by order of the Italian government in the early 1990s [Kaniewski et al. 1999], but its commercialization has been postponed, due to issues related to public acceptance in Europe. Commercial tomato lines that are resistant to tobacco mosaic and tomato mosaic viruses were generated, but never used in commercial settings.

Many other important crops, including rice, peanuts, wheat, barley, maize and others were generated with a range of degrees of resistance to a variety of viruses and field-tested around the world. Research to develop virus resistant cassava and sweetpotato for eventual use in developing countries is underway in several laboratories.

The primary benefit of cultivating virus resistant crops will be for farmers, with secondary benefits for consumers. Farmers will produce higher yields of better quality crops with less labor and chemical inputs (insecticides are often employed to reduce the population of insect vectors). Consumers will have access to more abundant, cheaper food, that is free of virus-induced symptoms and therefore generally considered to be of superior quality. Virus-free materials are often better suited for processing, transportation and storage. Furthermore, virus resistant crops will bring benefit for the environment because of reduced use of chemical insecticides.

## TECHNOLOGY ASSESSMENTS IN THE CONTEXT OF DEVELOPING COUNTRIES

Preventive measures are commonly used in modern agriculture to minimize losses due to pest and pathogens. In developing countries, the options available to the farmer to reduce crop losses and employ conservation agriculture can be very restricted. It is in these cases where natural or transgenic resistance to any kind of biotic stress can be of highest value.

Modern weed control will provide enormous benefits for farmers in developing countries, especially in tropics. At present hand weeding is the most time and labor consuming activity there and still not enough effective. Farmers in developing countries need access to modern weed control technology. To succeed they need affordable access to planting material, herbicide and sprayers, but more than anything else they would need education.

Transgenic insect and virus resistant crops are of immediate value, as the sole technology required are seeds or other planting materials. The technology is equally as

applicable to large and small farms, including subsistence farmers and there are no other costs or activities needed to take full advantage.

Insect resistant cotton is the best example of existing product used beside the US by small land-holding farmers in South Africa, India, Australia, Argentina, Mexico, Colombia and China. Poor farmers are enjoying increased yields, healthier environment and higher profits.

Transgenic virus resistance is the next most valuable trait for developing countries, because of the widespread impact of viruses in many tropical crops, and because the technologies are well established for many types of plant viruses. The use of existing virus resistant crops will require tests to determine if the resistance is effective against local virus strains. If existing crop varieties are not acceptable in the region, local varieties should be used after introducing resistance genes by breeding or by direct transformation. If local regulations do not accept genes from viruses that are not indigenous, local virus genes should be cloned and used.

At present several virus resistance projects are in advanced stages of testing and development in several countries outside of the US Potato cultivars that are resistant to potato viruses X and Y are ready for commercialization in Mexico, and a variety that includes resistance to a third virus, potato leafroll virus, is at an advanced research stage. China has developed tomato varieties with resistance to cucumber mosaic virus and selected lines of practical value. In Kenya, two years of field-testing was completed with experimental sweetpotato lines showing moderate levels of resistance to sweetpotato feathery mottle virus.

While a decision to use genes from local virus isolates vs. a non-local strain may be appropriate in some cases, it will likely delay commercialization of disease resistant varieties. Several developing countries have generated papaya that are resistant to papaya ringspot virus, some of which have developed their own unique gene constructs while others have selected to use genes developed outside of their specific regions. The second approach was not effective due to difference in local virus strain. Field tests of virus resistant varieties are well advanced in some of these countries. A cassava variety with moderate levels of resistance to African cassava mosaic virus is ready for field-testing in Africa. Groundnuts with engineered resistance to Indian peanut clump virus are being field tested in India. These limited examples indicate that transgenic virus resistance is well underway in many developing countries. Most of these projects are being conducted in cooperation with advanced laboratories of partner institutions, including those in industry sectors, non-for profit research institutes, and universities.

An important first step in accelerating the development and introduction of virus or insect resistant crops in developing countries will be the identification of major problems through epidemiological studies. This should be followed by development of the transgenic varieties, in cooperation with advanced laboratories as necessary. The country introducing the product should have (1) clear regulatory procedures that evaluate the new materials on a rational and science-based system; (2) a suitable seed propagations and distribution system; and (3) a likelihood of favorable public acceptance. Each of these conditions will require adequate preparation and transparency in the local situation, and may require that scientists be directly or indirectly involved in each step of the process.

#### THE FUTURE

In the near future, the current types of insect resistant crops will likely be accepted and adapted by farmers and consumers in a number of countries. Virus resistant crops that are developed by and for developing countries should reach farmers after field selection and crop registration. A number of projects that are presently in the research stage and new projects that target the protection of key crops against economically important viruses should be advanced as rapidly as possible. Good examples of such projects include maize with resistance to maize streak virus and maize rough dwarf virus, rice with resistance to rice tungro virus, wheat with resistance to barley yellow dwarf virus, cotton resistant to cotton leaf curl virus, citrus resistant to citrus tristeza virus, soybean resistant to soybean mosaic and bean pod mottle viruses and palms resistant to cadang-cadang viroid.

In addition basic research is needed to continue to develop mechanisms for conferring resistance to fungal and bacterial diseases and to plant parasitic nematodes. Furthermore, there is a great need to enhance the local capacity for developing and evaluating the biosafety issues that may be associated with certain types of disease resistant traits, so that new varieties can be released to farmers in a timely manner. Without an adequate and transparent approval process, it is likely that new crop varieties will not be available to farmers in developing countries in the near future.

### REFERENCES

Cann A.J., 1997. Principles of Molecular Virology. Academic Press, San Diego, CA.

Gressel J., 2002. Generation of biotechnologically derived herbicide-resistant crops [In:] Molecular Biology of Weed Control, Taylor and Francis, New York, NY, 219-277.

Honee G., 1999. Engineered resistance against fungal plant pathogens. Eur. J. Plant Pathol. 105, 319-326.

James C., Krattiger A., 1996. Global review of the field testing and commercialization of transgenic plants: 1986 to 1995. ISAAA Briefs.

James C., 2003. Global review of commercialized transgenic crops: 2002. ISAAA Briefs.

Jenkins J.N., 1999. Transgenic plants expressing toxins from Bacillus thuringiensis [In:] Biopesticides: Use and Delivery, Humana Press, Totowa, NJ, 211-232.

Kaniewski W., Ilardi V., Tomassoli L., Mitsky T., Layton J., Barba M., 1999. Extreme resistance to cucumber mosaic virus (CMV) in transgenic tomato expressing one or two viral coat proteins. Mol. Breeding 5, 111-119.

Kaniewski W.K., Lawson C., 1998. Coat protein and replicase-mediated resistance to plant viruses. In Plant Virus Disease Control, APS Press, St. Paul, MN, 65-78.

Kaniewski W.K., Thomas P.E., 1993. Field testing of virus resistant transgenic plants. Sem. Virol. 4, 389-396.

Kaniewski W.K., Thomas P.E., 1999. Field testing for virus resistance and agronomic performance in transgenic plants. Mol. Biotech. 12, 101-115.

Nelson R., McCormick S., Delanney X., Dube P., Layton J., Anderson E., Kaniewska M., Proksch R., Horsch R., Rogers S., Fraley R., Beachy R., 1988. Virus tolerance, plant growth, and field performance of transgenic tomato plants expressing coat protein from tobacco mosaic virus. Bio/Technology 6, 403-409.

Opperman C.H., Conkling M.A., 1998. Bioengineering resistance to plant-parasitic nematodes. In Plant Nematode Interactions, American Society of Agronomy Inc., Madison WI.

Powell-Abel P., Nelson R., De B., Hoffmann N., Rogers S., Fraley R., Beachy R., 1986. Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. Science 232, 738-743.

Rommens C.M., Kishore G.M., 2000. Exploiting the full potential of disease-resistance genes for agricultural use. Curr. Opin. in Biotech. 11, 120-125.

# UZYSKANIE ODPORNOŚCI ROŚLIN NA STRES BIOTYCZNY METODAMI BIOTECHNOLOGICZNYMI

Streszczenie. Stresy biotyczne powodują znaczące straty w produkcji wszystkich roślin uprawnych, zwłaszcza w krajach rozwijających się. Zapobieganie tym stratom, dzięki upowszechnieniu odmian odpornych, konwencjonalnych lub transgenicznych, byłoby tam łatwiejsze i tańsze niż wdrażanie klasycznych zabiegów rolniczych, takich jak stosowanie preparatów chemicznych. Niezwłocznie należałoby w tym celu wykorzystać odmiany odporne, już zarejestrowane w innych krajach, kontynuując jednocześnie badania ukierunkowane na wyhodowanie lokalnych odmian odpornych na szkodniki i choroby wirusowe. W przyszłości, jeśli to okaże się możliwe, badania te powinny objąć także odporność na patogeny grzybowe, bakteryjne oraz na nicienie. Warunkiem wstępnym realizacji wszelkich takich programów w krajach rozwijających się jest przyjęcie odpowiednich regulacji prawnych oraz ich akceptacja przez lokalną społeczność.

**Słowa kluczowe**: stres biotyczny, rośliny transgeniczne, odporność, chwasty, szkodniki, wirusy, grzyby, bakterie, nicienie

Wojciech K. Kaniewski, Principal Scientist, Plant Molecular Virology, Product & Technology Cooperation, Monsanto Company, 700 Chesterfield Village Parkway, St. Louis, MO 63198, e-mail: Wojciech.K.Kaniewski@monsanto.com

Prof. Roger N. Beachy, Plant Science Center, 975 North Warson Road, St. Louis, MO 63132, e-mail: rbeachy@danforthcenter.org