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Research Challenges and Needs for Safe Use of Transgenic Organisms: Introduction

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Fifteen years have passed since the first transgenic plants were reported in 1983. Since then we learn about a new transgenic organism almost every day. We also could state that a new epoch has been started. The first transgenic tobacco plants were quickly followed by many other crops bearing agriculturally important new traits. Most of these plants are already in fields and have proved their superiority over their sister nontransgenic cultivars. Genetic engineering has become the most powerful technique for improving crops for an environmentally friendly sustainable agriculture. Using transgenic plants significant reduction of pesticide treatments have been achieved and alternative solutions be envisaged for integrated pest management systems, too. Till today, approximately 25000 transgenic crop field trials were performed globally and transgenic plants were grown over 12,8 million hectares only in 1997 (James 1997). Scientists are estimating that this figure will be four times higher in 1998. The use of transgenic plants has entered into a logarithmic phase. In 1998 more than 35 percent of planted soybeans are transgenic in the US, while more than 25% percent of canola are transgenic in Canada this year, 25% of 1998 US corn crop is transgenic. Why do we need the use of genetic engineering? Conventional plant breeding can not compete with this fast precision plant breeding and we can not wait. The world population continues to grow at about 1,5% a year. It is projected to reach 6 billion in the coming year and UN statistics are estimating 8 billion for 2020. Nowadays contrary to the advanced agriculture and the extensive use of agrochemicals more than 40 % of the crop productivity is lost due to the competition with weeds, to pests and pathogens (Vasil 1998). Additional loss is attributed to the postharvest period. This could reach a very high figure in developing countries due to the lack of advanced storage facilities. The challenge is here, because we have to double our food production on less per capita land, with less water, and under non-adequate environmental conditions (Vasil 1998). The introduction of this new technology is welcomed almost all over the world. But some concerns are expressed because in the past, as a consequence of industrial revolution incidents resulted in environmental damages and occasionally loss of life. Biotechnology has been used for centuries during the selection of higher yielding plants, microorganisms and animals by breeding for particular phenotypic characteristics. This technology has been accepted and approved by different societies and concerns over safety has never been a major problem. Schell (1993) elegantly demonstrated the relations among the development of plant breeding, predictability and experiences (Fig. 1.).

In conventional breeding two whole set of genome recombine and often the product contains no useful traits. With *in vitro* techniques, much less genetic material is used, than in traditional breeding. In precision plant breeding the complete primary structure of the engineered gene is known. In conventional breeding when we have several

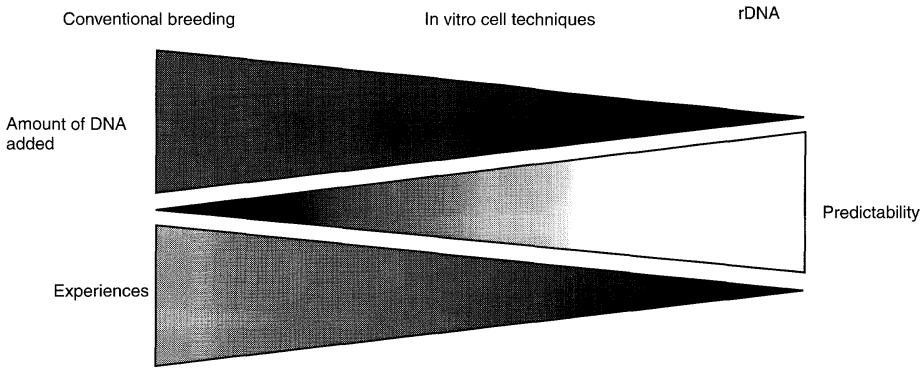

Figure 1

hundred years of experiences breeders still prejudging the product of the cross, while in the case of genetic engineering the desired trait will almost always be expressed. In this later case however our experiences are limited to results achieved over the past decade. This is mostly the basis of the concerns. However we have to emphasise that genetic engineering could be more safe than conventional breeding, when the breeders are crossing wild-type plants (with unknown traits) with cultivated breeding lines to increase biodiversity in agroecosystems. During this very short period eye catching results are achieved mostly in the field of the plant protection area. This is plausible, because public awareness on the extensive use of pesticides gave priority to work out alternative technologies for reducing the use of pesticides. This important goal brought its first results, the virus resistant crops, the insect resistant major crops like cotton, potato, corn, and very recently fungus resistant plants. Over an extremely short period of time the technologies are rapidly evolving and improving day by day. I will use the examples of virus resistant and insect resistant plants to give a short introduction on how changes of the initial techniques are good examples, how scientists improving genetic engineering for the safe use of transgenic organisms.

VIRUS RESISTANT TRANSGENICS

One of the most striking results came in the field of plant virus resistance. Till the epoch of transgenic technology only laborious and slow resistance breeding produced temporary solutions to protect our crops from devastating virus epidemics. Genetic engineering made it possible to use viral sequences against the superinfecting viruses. The well known "cross protection" phenomenon in plant pathology i.e. a mild or attenuated strain of a given virus could produce protection against a related pathogenic strain, has been utilized to engineer protected plants. It was shown as early as in 1986 that integrated coat protein (CP) gene of tobacco mosaic virus (TMV) could led to protection (Powell Abel et al. 1986). This initial success was followed with other examples using a series of host-virus systems. Nowadays more than a hundred host-virus combinations are in an advanced stage of R and D for commercialization. In China above, hundreds of hectares are planted with transgenic virus resistant tobacco and tomatoes. Besides the CP approach, several other virus gene/s proved to be good candidates for engineering virus resistance, namely replicase gene, movement

protein, helicase etc. (Wilson 1993). There are no indications or data to show that this technology has/had any negative impacts on the environments. Scientists raised several questions to be answered before this plants are globally introduced. Three main points were raised: (i) the potential for hetero/transcapsidation when superinfecting viral RNA interact with the transgenic CP. (ii) recombination between the transgenic CP gene transcript and the superinfecting viral RNA, and (iii) synergistic effect of the transgene. Heteroencapsidation and/or transcapsidations are well demonstrated in natural infections, because in nature almost universally more than one strains or sequence variants are present in a diseased plant (in Matthews 1992), due to that fact that viruses are quasispecies. So the frequency of getting hetero/or transcapsidations is not higher than in the natural conditions. It is important to note also that in a resistant plant no virus replications could be detected or very limited compared to a susceptible host, where the virus replication is enormous can reach easily 1% of the total fresh weight of the leaf tissue. Due to the improvement of the CP mediated protection technology, today a truncated CP gene also could be used for engineering resistance. In this case the truncated CP could not encapsidate the viral RNA so hetero or transcapsidation could not occur avoiding the possibility of the virus movement to a non host plant via aphids. The most important question concerns the potential for recombination between the transcript of the transgene and the virus RNA. The recombination between virus RNAs could be detected in several virus-host comminations, and could reach up to 35% (in Tepfer and Balázs 1997). This recombination is rarely manifested in the evolution of a new virus strain, but definitively recombination is part of the virus evolution. It was indicated above that in a resistant/transgenic plant, virus replication is restricted close to zero, so the chance to get recombination between transgene transcript and viral RNA can be discounted.

Regarding synergistic effect, when the expressed transgene could lead to symptom worsening the disease symptoms, these are limited to the individual plant. As no genetic change occurs the phenomenon has low impact. However a detailed further research is necessary to answer the exact impact of this phenomenon on the environment and on the envisaged use of transgenic coat protein mediated virus resistance technology.

INSECT RESISTANT CROPS

The extensive use of pesticides resulted in the appearance of insecticide resistant population of the target insect. Today in Europe almost all population of potato beetles developed a certain level of resistance and even combination of the insecticides are having less and less effective protection on crop plants. Entomologists are expecting that in a few years no currently used insecticides will be available to control this insect. The insecticides had a negative impact also on beneficial insects by reducing their populations, too. In this case genetic engineering also envisaged an alternative possibility and initially insect control was achieved by expressing the insect control genes of *Bacillus thuringiensis* (known as Bt). Bt is a naturally-occurring soil bacterium which produces a s endotoxin lethal to specific insects. Depending the Bt strain the toxin produced selectively kills lepidopteran larvae, coleopterans or dipterans. Bt protein has no toxicity to beneficial insects, other animals and humans. The mode of action of Bt also well described and known that the protein involves the disruption of K^+ transport across brush border membrans of the susceptible insect. As it binds to specific receptors, the specificity of the given Bt reasonably high. The first transgenic plants expressing Bt genes were sufficiently protected from the target insects. As entomologists find out the insects are developing resistance against a pesticide quite rapidly, some scepticisms emerged in using this transgenic approach, too. To overcome an this problem, Bt genes were modified and, multicopy and mutated Bt together give less chance to get resistant insect population to the different

integrated insect control Bt genes. Very recently – after a detailed study of the protein structure - the three insecticide active protein domains were synthethised from three different toxin protein genes resulting a hybrid protein and minimazing the development of a resistant insect. Field tests of potato, cotton and maize confirmed the greenhouse results. Protection was equivalent to weekly insecticide spraying. Besides the integrated Bt protection effect, other genes are also being used for engineering insect resistance. The major goal of these research are (i.) avoid or minimize the development of resistant insect population. (ii) maintain and prove the target specificity, (iii) no insecticidal effect on beneficial insects.

In summary transgenic technology is offering an effective alternative to crop protection, too. The constant development/improvement of these methods make their use safer and better. Of course, the raised scientific questions has to be answered and carefully studied, before large-scale introduction taken place. In this early phase of introduction regular monitoring seems to be important to evaluate the effectivity and the impact of this transgenic technology both on agriculture and on the environment.

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