Kragujevac J. Sci. 23 (2001) 127-136.

GENETICALLY MODIFIED PLANTS - ASSESMENTS AND PERSPECTIVES -

Desimir Knežević, Veselinka Zečević, Dušan Urošević, Vesna Urošević, Biljana Dimitrijević, Danica Mićanović

Agricultural Research Institute of Serbia- Belgrade, Center for small grains Kragujevac, Save Kovačevića 31, 34000 Kragujevac

(Received March 31, 2001)

ABSTRACT: Creation of new genotypes can be realized by using conventional plant breeding and recently by using new biotechnology. Classical breeding technology represents long-term process of genotype creation. New biotechnological methods make possible to overcome this problem and develop transgenic plants for specific utilization. Application of new technology makes it possible to transfer gene to the crop plants by genetic manipulations encoding the desirable traits and developing new genetically modified plants. In this review paper data on research of genetically modified organisms, development of new technology in plant breeding and possibility of its application and intellectual property rights protection in plant breeding will be presented.

INTRODUCTION

Plant breeders have made a major improvement in plant yield potential and quality, pest and diseases resistance. The yields of the major crops have doubled on the base of plant breeding programs. The plant breeders have made a major contribution to increased yield potential, increase pest/disease resistance and improved quality through breeding and selection of new cultivars. Appart the classical methods of plant breeding, also the biotechnological methods of genetic diagnostics, tissue culture and genetic modification have been developed to a point to make further improvements in the crop quality and yield potential. The assessment of contribution of this new technology in plant breeding is very difficult. It is also difficult to forecast when this new technology will have a major impact on the development of commercial products in a wide range of crops. However, the plant breeders can achieve this new technology for assessment and development of commercial products in a wide range of crops. The first succesiful gene transfer has been realized in monocotiledonous rice plants (De la Pena et al., 1986), maize (Ohta, 1986). Which of these scientific and technical advances will translated into new cultivars depends on lot of such factors: the industry structure, technical progress, the scope of proprietary protection while the regulatory system is also very important (Konstatinov and Mladenovic-Drinic, 1998; Knezevic et al., 1998; Wiegand, 1998). Biotechnology can assist plant breeders also through the increase of genetic variability. The use of this technology is currently developing while the transformed plants and products of genetically modified plants need to be registered and tested.

PLANT BREEDING

Conventional breeding involves making a large number of crosses between divergent parents, which have been chosen by the breeder because of their desirable characteristics. During the selection cycles, the breeders selected new genotypes which have a combination of desirable characteristics and which are different from other cultivars. Creation of a new cultivar by using a classical method of crossing can take up to 10 years. Realization of a commercially new cultivar takes at least another 5 years. The conventional plant breeding is characterized by a lot of problems: long-term plant breeding programs, expenses during the cultivation and analyses of a great number of plants which take both time and space. The conventional breeding has a complex task to develop new genotypes with a high yield potential, best quality, and a high environment, adaptability.

The breeders have recently accepted biotechnological methods and techniques to overcome problems of the conventional plant breeding. Biotechnology can assist a plant breeder to improve crops by increasing the efficiency and effectiveness of a selection on the one hand, and by broadening the genetic base for selection on the other. Over the last few decades technical advances in the recombinant DNA, and tissue culture methods have developed to have a major impact on the efficiency and success of the conventional plant breeding. The restriction fragment length polymorphisms (RFLPs), the proteinase chain reaction (PCR) or other new methods of genetic selection can improve effectiveness of the selection in a plant breeding process. These methods allow a selection at the level of genotype and show advantage of characteristics, which is difficult to assess at the level of phenotype in the field as resistance to diseases. The techniques for increasing the efficiency of a selection include the anther and pollen culture, producing true-breeding homozygous lines more rapidly than using pedigree-breeding or single-seed descent. Genetic transformation and protoplast fusion are techniques for broadening the genetic base for selection. These techniques lead to changes in genetic composition of the plant and the new plant cultivar needs to have a certificate of a safe plant. The cultivars developed by using of such techniques are known as genetically modified organisms (GMOs). The cultivars developed by conventional plant breeding and their products are generally recognized as safe. However, those conventionally bred cultivars of crops, which are new in a particular country, may be required to have on authorization for commercial production.

There have been new scientific approaches in identifying and isolating genes which confer characteristics such as diseases resistance, virus resistance, insect resistance, herbicide resistance and male sterility (Friedt, 1998). The protoplast fusion was applied in the transfer of male-sterile cytoplasms, in the production of hybrid seed in rice and oilseed rape and on the disease resistance in tomato and potato. An intensive research of manipulation used for the crop improvement has been carried on the major field crops such as: wheat, maize, rice, barley, sorghum, sugarbeet, potato, tomato, sunflower, alfalfa, cotton. Most of these crops experienced a successful transformation. Also, for some of them, field tests of genetically modified crops, which are potentially new varieties, have been carried out. The transformation of wheat and barley was not as successful as with the other crops since the transformation techniques, which have been reported for other crops, still need to be improved.

The mentioned crop plants are used in food industry. However, the crops can be used for other purposes, not only as food for people and animals. Therefore, the plants producing oil are used in molecular farming (manufacturing of plastics and proteins for pharmaceutical purposes). Some of transformed crop plants possess genes used for human serum albumin and mammalian antibodies, while research activities in biotechnology can lead to a decrease in production costs of therapeutic agents. The pharmaceutical products from GMO plants are usually competitive at the market and the buyers are satisfied, but regulatory issues could be very different (Connett et al., 1992). The plant biotechnology, make it possible to develop plants for specific utilization. This is the reason why big investments are made in oil plant biotechnology research for improving of crops to satisfy demands of existing markets. Many of characteristics, which have been transferred to the crop plants by the genetic modification, are connected with the improvement of agronomic characteristics, which are of a primary value to the farmer.

Genetically engineered soybean, cotton, rice, oilseed rape, corn, sugar beet and alfalfa are expected to enter the market by the year 2000 (Oxtoby and Hughes, 1990). Methods and approaches to a transformation of other species are slowly developing because of the limiting factors as well as lack of routine and reliable methods for the plant transformation and the limiting availability of suitable single dominant genes. Once a gene is identified and isolated it provides a base for breeding of transformed plants. A method of production of commercial cultivars depends on how successful the propagation of species is. There are differences in propagation methods, for example, tubers are used in vegetatively propagated crops like potato, while seed crops varieties are produced by either selfing plants or the plants homozygous for the introduced genes. Development of new varieties of genetically manipulated plants requests answers to many question regarding the effect on environment and the risk to consumers. These releases are in charge of the authorities in UK it is Advisory Committee on Releases to Environment (ACRE). There have been many field experiments in which genetically transformed plants of species are used for examining the effects of plants growing in the field and for examining the risk of gene transfer between genetically manipulated crops and their untransformed plants or plants of relative species. The risks are associated with the newly inserted gene and how it will behave in its new genetic plants.

The first publication about transgenic plants appeared in 1984 (Horsch et al., 1984) and it marks the beginning of exceptionally rapid progress directed to the application of this new technology in the crop improvement. The use of genetically engineered crops is an entirely new and untested technology. The transfer of transgenic plants from the laboratory to the field is improved by a cautions, step by step, approach. The applications of the plant genetic modification is at a threshold and commercial organizations are starting to outline precise plans for field test and marketing of GMO crops as products and for the protection of the product innovation and complying to the regulation. Commercial researchers who work on developing GMO crop plant products specified several tiers of official examination and approval:

a) laboratory and glasshouse research; b) submitting for patent protection; c) regulations on release to environment of GMO crops; d) cultivar protection and registration; e) regulations on GMO crops, used as food.

Laboratory research regulations referring to the plant genetic manipulation in research laboratories are becoming less strict as the experience has grown and the excellent safety results have been recorded. The other levels regulation of concerning the protection and regulatory approval of GMO cultivars are extremely complex and considerably vary from country to country. Eleven developed countries cover about 90% of the annual retail seed consumption value (USA, Canada, Japan, Australia, France, Germany, Denmark, Holland, Italy, Spain and UK) (Kidd, 1985), while Western Europe and the USA lead in the development and application of crop biotechnology.

PLANT GENETIC TRANSFORMATION

Genetic transformation can be defined as the transfer of foreign genes isolated from plants, viruses, bacteria or animals into a new genetic system. Successful genetic transformation requires the production of normal, fertile plants, which express the newly inserted genes. The process of genetic transformation characterized a several distinct stages, namely insertion, integration, expression and inheritance of the new DNA. Methods of gene insertion can release by the use of bacterial (Agrobacterium species) or viral vectors and direct gene transfer (DGT). These techniques utilize similar gene constructs, comprising bacterial or viral promoters linked to appropriate genes. The precision with which Agrobacterium transfers its DNA into the host plant’s genome makes it an ideal method for genetic transformation. Direct evidence of the insertion of T-DNA, using molecular techniques has been obtained in different species of plants (Rainer et al., 1990). The T-DNA from Agrobacterium can therefore be integrated into and expressed by the genome. Further refinements of methods of DNA delivery by

Agrobacterium species require sophisticated methods to permit rapid identification of successful transformation. Agroinfection magnifies such events, permitting even a single transformation event to be visualized as symptoms of viral disease in the whole plants. Maize has been successfully transformed with the maize streak virus (MSV) while wheat with the wheat dwarf virus (WDV) using Agrobacterium as a vector. The viral genome inserted between the T-DNA borders of *A. tumefaciens* did cause infection in the agriculturally important cereal crops, wheat (Dale et al., 1989; Marks et al., 1989). Wheat seedlings grown in vitro and rate of infection with WDV varied not only with the strain of Agrobacterium but also with the bacterial species.

PROTECTION OF TRANSFORMED PLANTS

The intellectual property right (IPR) is a regulation, which exists in developed countries which defined patents, trademarks, trade secrets, copyright and plant cultivar rights (plant breeders' rights). It is known that 10 years are approximately needed to approve new plant cultivars containing a patented gene. Also, another 10 years are necessary to obtain a patent protection. Intellectual property rights protection folows particular difficulties in the field of plant breeding (Bent et al., 1987). These difficulties appear because many technical advances are incremental and often are not able to satisfy the standard criteria for patent protection. There are also other difficulties connected with a great complexity of biological systems.

Application of IPR protection is different among the countries. Some countries developed a specific system of IPR protection which is called the plant variety rights (PVR) system. This system protects innovations while the patent system protects inventions, which may or may not be verified as innovations. Target for protection defined all genetic materials and information associated with plants, namely advanced breeding lines, varieties, cloned genes, probes (Konstatinov and Mladenovic-Drinic, 1998).

The PVR system is in compliance with the International Convention for the Protection of New Varieties of Plants (UPOV-Union International pour la Protection des Obtentions Vegetales) which allowed control of seed production, processing and sale of approved cultivars and could also be extended by a national law legalization to products obtained from a harvested cultivar. The potential cultivar must be tested in field at least several years as well as chemically analyzed in order to establish its 'distinctness, uniformity and stability' (DUS). The (DUS) criteria show that a potentially new cultivar is different from other varieties. In Europe, there is a developed system for testing the value for cultivation and use (VCU). The seeds of cultivars which meet the criteria of DUS and VCU are enter the "National List" and can be sold legally. The breeders can use such cultivars for the multiplication and breeding program. According to the UPOV Convention, the plant breeders can freely use registered plant cultivars for the plant-breeding program. There are some restrictions referring to direct use of cultivars as parents in the hybrid seed production and small changes such as that a cultivar is essentially derived from another one. One of important provisions is that a national government give a possibility to farmers to retain the seed of a crop for replanting, this is called the "farmer's privilege".

Process of producing new plant cultivars through a conventional plant breeding is different from obtaining products from biotechnology research (gene controlling of agronomic important traits) could be used in the production of cultivars, species etc. This confirms that such new plants are results of an inventive step which has a wider possible application than conventional plant breeding which is limited by crossing incompatibility. A patent is a form of property right granted by a state authority in respect of inventions of its author against unauthorized use by others. To obtain a patent protection, several criteria must be met. The protection given by a patent is determined by the scope of the 'claims', which are legal definitions of the technical products or invented process. There are strong reasons to suppose that criteria for obtaining a patent protection can be met. The types of inventions in this field for which patent applications have been filed include: tissue culture methods, fusion protoplast methods, gene insertion methods, vectors, isolated genes and the "gene promoter" (Connett et al., 1992). There are differences in the interpretation of this patent, "gene promoter" means a promoter which is active in a plant or one which is derived from the plant.

The PVR and patent system for legal protection of GMO and plants contains contradictory resolutions in several aspects. This conflict could be resolved by passing a new regulation specifying the patent rights of a patented gene, which appear in registered cultivars. Another possibility is that patent holders should be forced to grant a licence to plant breeders the "licence of right" to use the patented process or product of a cultivar in the production of a new cultivar. An EC directive on the legal protection of biotechnological inventions resolves this. The directive confirmed the availability of patent protection for plants, plant parts and plant materials, but excluded plant cultivars from the patent protection (Leskien and Flitner, 1997; Knezevic et al., 1998).

DEVELOPMENT AND RELEASE OF GMO IN THE ENVIRONMENTAL CONDITIONS

The authorities all over the world have generally taken the view that release to the environment of products derived from certain techniques should be subjected to a specific regulation. This regulation defines the products, processes and techniques to be in the genetic modification. Plant breeding by crossing different genotypes represents "random" genetic modifications with rare environmental impact. The regulation of environmental safety of GMO (Regulation EC No. 258/97 and 90/219/EC) is mainly based on the phenotype of the plant rather than on its method of genetic construction. Since its reaction on the environment is unknown it is necessary to assess GMO during the multiplication and answer the following questions: a) is GMO more persistent in the environment; b) do GMO plants or other plants to which the favorable gene has been transferred have an additional characteristics which could be responsible for the adaptation to the environment; c) does the genetically modified plant with the inserted gene transferred by pollen or by any other similar way become more persistent or invasive.

The products of biotechnology, in most cases, are safe and not significantly different from those which are obtained in conventional plant breeding. The procedures for developing genetically modified plants need to be identified, tested and improved to the level to minimize the potential risks. The organizations such as the US National Research Council and UK Royal Commission on Environmental Pollution have developed regulatory procedures, although have already decided how to regulate the environmental release of GMO plants in the best possible way. The releases of GMO plants into the environment have been carried out in a lot of countries, which have their own developed procedures and policies for regulating GMO, field tests. The Organization for Economic Cooperation and Development (OECD) has established common regulatory criteria and procedures, which can be adopted, by its member states. The OECD recommends that GMO applications should be assessed on a case-by-case basis by an expert group, and the development of GMO products should be carried out and move from the laboratory to the growth chamber, to the greenhouse and to limited field testing. In USA, estimation of environmental safety of genetically modified plants is under the jurisdiction of the United States Department of Agriculture (USDA) and Environmental Protection Agency (EPA). These organizations have prepared guidelines for the GMO field tests.

Having obtained the permission to grow GMO plants in the field and the evaluation of their properties, the breeders need to decide whether the registered GMO will be used in the breeding program for crossing. The breeders will be able to combine a particular trait conferred by the inserted gene with the other qualities of its best available germplasm in a potentially new cultivar. The next question to be answered is whether or not the registration will involve safety assessment of the use of the plant product for food. The major tasks are to develop methods of environment impact assessment for genetically modified plants, and to assess and regulate at an early stage the safety of GMO crops as a food.

The regulatory system pertaining to the use of plant products of GMO crops for food has been established in developed countries. In the USA laws and procedures regulating use of GMO crops is under the control of FOOD and Drug Administration (FDA) while in the European Community such regulations are currently a responsibility of its member states.

PERSPECTIVES

Plant genetic engineering offers great benefits to the environment, by replacing the present sprayings of crops with herbicides, fungicides and pesticides with a combination of inherent engineered resistance to pests and diseases, and selective treatments with specific 'safe' chemicals. Also, various types of genetic engineering can make very major contribution to reduce crop losses due to insect pests, plant diseases, and competing plants, both in terms of yield loss and reduction in food quality. Protections of crops from their insect pest have been realized by genetic engineering. Plant-derived insect-resistance genes and transferring them to other plant species by genetic manipulation make possible to produce crops with significantly enhanced levels of resistance. Creation of GMP - transgenic crop plants with resistance to pathogens it is necessary to identify 'resistance genes' to develop some understanding of the mechanisms of the interaction between the resistance factor and target pathogen. Production of GMO - transgenic crops with resistance to, or tolerance of, herbicides is more controversial application of this technology. Unlike the examples of insect resistance and disease resistance this is not a substitution technology, but rather may increase the dependence on exogenously applied synthetic chemicals. There are arguments that can be advanced to support the suggestion that the selective use of herbicide and herbicide resistant cultivars may be major improvements on current practice for controlling weeds in some situations.

Nowadays is well documented that classical methods of plant breeding can be supplemented and complimented by genetic transformation, which allows the transfer of genes across species, irrespective of taxonomic relationships, and thereby overcomes one of the major limitations of plant breeding. Advances in cellular and molecular biology in recent time have made it possible to introduce defined genes into plant cells, and regenerate plants in which the transgene is expressed and transmitted to progeny as a dominant Mendelian trait. All the major crop species (cotton, oilseed rape, maize, wheat, barley, oat, rye, sugarbeet, potato, tomato, rice, soybean etc) have been transformed and some are already being cultivated commercially GMO in China and the USA.

Transformation technology is readily available to plant breeders decisions will have to be made on what genes to transfer to modern plant cultivars. A breeders will have to determine which genes are available for successful transfer, which genes are going to stimulate the least amount of controversy with regulatory officials, the cost to obtain approval and which genes are going to equip a cultivar with high marketing value to pay for the research effort. Once a superior transgenic plant is found, breeders will probably use conventional breeding procedures to introgress the transgene into new cultivars. The procedure would probably be more efficient and less costly than having to subject all-new transgenic plants to a safety and environmental assessment. The new transgenic plant cultivars will also have to satisfy regulatory officials that it does not pose any threat to man, his domestic animals, and the environment or to the orderly marketing of conventional plant species cultivar.

Genetically modified plants cannot be excluded from improvements of regulations governing transgenic experimentation, since the technology must be proved to be safe before it can be applied in situations where the public may come into contact with it. Genetic modification has great potential to contribute to more sustainable and environmentally sound agricultural system and to provide renewable sources for industry. Therefore scientific community, industry and governments on the base of legal protection and regulatory systems can offer the choice of products improved through genetic modification.

References

[1] Bent, S.A., Schwaab, R.L., Collin, D.G., Jeffrey, R.D. (1987): *Intellectual property rights in biotechnology worldwide*. Stockton Press, New York.

[2] Connett, R.J.A., Barfoot, P.D. (1992): *The development of gnetically modified varieties of agricultural crops by the seed industry*. In: *Plant genetic manipulation for crop protection* (Eds. Gatehouse, A.M.R., Hilder, V.A. and Boulter, D.). pp. 45-72.

[3] De la Pena A., Lorz, H., Chel, J. (1986): *Transgenic rice plants obtained by injecting DNA into young tillers*. Nature, 325, 274-276.

[4] Dale, P.J., Marks, M.S., Brown, M.M., Woolston, C.J., Gunn, H.V., Mullineaux, P.M., Lewis, D.M., Friedt, W. (1998): *Genetic modification of crop plants - recent achievements and future perspectives.* Proceedings of 2nd Balkan Symposium on field Crops. (S. Stamenkovic, ed.), 1, 9-11.

[5] Horsch, R.B., Fraley, R.T., Rogers, S.G., Sanders, P.R., Loyd, A., Hoffman,H. (1984): *Inheritance of functional foreign genes in plants*. Science, 223, 496-498.

[6] Kemp, J.M.,Kemp, J.M., Chen, D.F., Gilmour, D.M., Flavell, R.B. (1989): *Agroinfection of wheat: inoculation of in vitro grown seedling and embryos*. Plant Science, 63, 237-245.

[7] Kidd, G.H. (1985*): The new plant genetics: restructing the global seed industry*. In: The world Biotech Report 1, Online, London, pp. 311-321.

[8] Knezevic, D., Zecevic, V., Marinkovic, I., Konstatinov, K., Mladenovic-Drinic, S., Andjelkovic, M., Micanovic, D. (1998): *Prospect and problems of genetically modified cultivars, development and impact on conventional plant breeding.* Proceedings of International Symposium Breeding of Small Grains, pp. 103-110.

[9] Konstatinov, Lj. K., Mladenovic-Drinic, S.D. (1998): *Biotechnology in intellectual property protection in breeding.* Proceedings of 2nd Balkan Symposium on field Crops. (S. Stamenkovic, ed.), 1, 157-160.

[10] Leskien, D., Flitner, M. (1997): *Intellectual Property Rights and Plant Genetic Resources: Options for a Sui Generis System.* Issues in Genetic Resources, 6.

[11] Marks, M.S., Kemp, J.M., Woolston, C.J., Dale, P.J. (1989): *Agroinfection of wheat: a comparison of Agrobacterium strains*. Plant Science, 63, 247-256.

[12] Ohta, Y. (1986): *High efficiency genetic transformation of mize by mixture of pollen and exogenous DNA*. P.A.N.Sci. USA, 83, 715-719.

[13] Oxtoby, E. Hughes, M.A. (1990): *Engineering herbicide tolerance into crops*. Tibtech, 8, 61-65.

[14] Rainer, D.H., Bottino, P., Gordon, M.P, Nester, E.W. (1990): *Agrobacterium mediated transformation of rice (Oryza sativa L.).* Bio/Technology, 8, 33-38.
[15] Wiegand, R. (1998): *Plant genomics: new approach to crop improvement and food quality enhancement.* XVII International Congress of Genetics, Beijing, China, Aug. 10-15