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Assessing ecological aspects of biosafety of genetically modified crops to the environment

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Abstract: Biotechnology alongside the introduction of genetically modified (GM) crops is constantly providing new opportunities for increasing crop productivity and tackling problems in agriculture, such as diseases, pests and weeds, abiotic stress and nutritional limitations of staple food crops. Crops possessing new traits enabling their use in pharmaceutical production are also being generated. As GM crops are being introduced into various locations characterized by different ecosystems, agriculture, biodiversity and agricultural practices, a scientifically based understanding of the environmental effects of GM crop cultivation would assist decision makers worldwide in ensuring environmental safety and sustainability. The main important environmental assessment of GM crops deals with their putative invasiveness, vertical and/or horizontal gene flow, effects on biodiversity and the impact on other products. These investigations are all highly interdisciplinary and complex. This paper deals with some of the most important problems related to the introduction of GM crops into the environment, such as plant protection, ecological effects of HRCs, gene flow,

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biodiversity, stress, ecological risks of Bt crops, effects on soil ecosystems etc. There is a clear need to further assess the severity, magnitude and scope of risks associated with the massive field deployment of transgenic crops. When assessing GMC interrelation with the existing cultivars, an increased knowledge base underpinning the development of GMC will provide greater confidence in plant science while assessing the risks and benefits of releasing such crops.

Key words: genetically modified crops (GMCs), environment, plant protection, HRCs, HTG, gene flow, biodiversity.

Introduction

Genetic manipulations have been commonly used throughout modern plant selection, including new gene combinations, the artificial manipulation of chromosome number, the development of addition and substitution lines containing specific chromosomes, chemical and radioactive treatments aiming to induce mutations and chromosomal rearrangements, cell, tissue and embryo cultures, in vitro fertilization and fusion of protoplasts for detection of interspecies and genus hybridization. The integration of these technologies has made the greatest contribution to genetic improvement in yield, adaptation to the environment (Dale 2002), resistance to pests and parasites (Beringer 2000), as well as quality improvements, as permanently required by both food producers and consumers (Dunwell 1998, Kuiper et al. 2001, Simic et al. 2005).

Developments in molecular biology and genetic engineering enabled the efficient modification of cultivated plants (Daniell 2002; Glisin 2005). These technologies may have an adverse impact on the environment (Conner et al. 2003) and human health and may increase poverty. In the forthcoming period it is necessary to devote more attention to the commercially and economically justified use of GM crops in agricultural food production (Pretty 2001, Nishiura et al. 2002; Boskovic et al. 2004a, 2010a, 2011). In this respect, linking science and politics should facilitate an overall assessment of acceptability and spread of GM crops. There are still significant disagreements about the extent to which sustainability, globalization, ethics and socio-economic approach should be parts of the risk assessment of GM crop use in most countries (Marshall 2002; Marshall and Moonen 2002, Perry et al. 2003; Simic et al. 2005).

Plant protection and genetically modified crops

Major plant protection considerations about GM crops (GMCs) refer to the potential incorporation of resistance genes into insects, fungal and bacterial pathogens and viruses, with long-term resistance as the main problem (Boskovic et al. 2000). Plant protection against pests through genetic modification is demonstrated by the use of Bt toxins from *Bacillus thuringiensis,* successfully used as spray for many years and introduced in a number of plant species such as: tomato, tobacco, cotton, etc. Pea lecithin has been proven to protect against insect attack in transgenic potato and tobacco. Trypsin inhibiting protein of fodder pea has also been used. GM plants provide many potential benefits to the environment by reduced pesticide use through the development of pest resistance. However, these advantages can be quickly denied: there are possible strategies to reduce the exposure of pests to transgenic products, thus lowering resistance levels and applying restricted transgene action (Sharma et al. 2000; Bouchard et al. 2003).

It has been shown that the application of transgenic resistance to viruses through indirect protection of *cap*-proteins is possible, and it can be used as a method for a wide range of viruses and hosts, e.g. expression of TMV *cap*-proteins for tobacco mosaic virus of potato and tomato resistant to PVX and PVY (Daniell 2002).

The use of genetic modification in fungal and bacterial plant pathogen control has also been developed. As with pests, the main problems are transient resistance and complex interactive pathogen-host relations. There has been an attempt to improve such resistance by the use of GM plants. In addition, there is a need to develop multiple resistance strategies (i.e. *pyramiding resistance genes*) towards different virulence levels of plant parasites (Daniell 2002, Bouchard et al. 2003; FAO 2003; Bošković et al. 2004a; 20004b; Bošković and Bošković 2009).

Horizontal gene transfer (HTG)

Horizontal gene transfer (HTG) is the transfer of the genetic material between cells or genomes that belong to different species, during processes that differ from common reproduction. During basic reproduction processes genes are transferred vertically from parents to their offspring. Bacteria are known to participate in gene exchange between different species in nature. It is performed via three mechanisms: during conjugation when the genetic material passes between cells, by transduction where the genetic material is transferred from one cell of infective viruses into another and by transformation in which genetic material is taken directly from the cell or its environment (Daniell 2002). For horizontal gene transfer to be successful, the foreign genetic material must become part of the cell genome, or be stably maintained within the recipient cell.

In certain cases, foreign genetic material that enters a cell will be removed before it incorporates into the genome, especially if it originates from another species. Under specific yet not fully clarified ecological conditions, foreign genetic material avoids the removal and incorporates into the genome.

Horizontal gene flow is well known in bacteria, but also in some higher plants and animals. In its base, this process comprises the whole biosphere, with bacteria and viruses acting as intermediaries in gene flow, gene pool, replication and recombination (Conner *et al*. 2003; Snow *et al*. 2004).

There are many potential pathways for horizontal gene transfer in plants and animals. As there are many viruses that infect plants and animals, transduction is expected to be the most frequent way of transfer. The latest investigations in gene therapy indicate potentially high importance of transformation for mammal cells, including humans. Direct transformation is not as significant for plant cells that have a protective cell wall. However, soil bacteria belonging to the genus *Agrobacterium* are able to transfer the T (tumor) segment of its tumor-inducing (Ti) plasmid into plant cells in the conjugation process. This Ti-DNK is widely used as a gene transfer resource in plant genetic engineering. Foreign genetic material from insects and arthropods with strong mutagenic reactions can also be built into plant and animal cells. Additionally, bacterial pathogens that penetrate into plant and animal cells can take up foreign genetic material and carry it into cells as vectors, probably to any kind of organisms on the planet. The most significant barriers to horizontal gene flow are employed after penetration of the foreign genetic material into the cell.

However, viruses and other genetic parasites, such as plasmids and transposons, have special genetic signals and probably a structure that enables them to avoid annihilation. The genetic material of viruses is protected by a protein coat. They discard their protein coat when penetrating the cell, thus enabling the creation of numerous copies or pass directly into the cell genome. Plasmids are *free fractions* usually round in shape so that genetic material can finally be maintained in the cell separately from the cell genome. Transposons (*jumping genes*) are blocks of genetic material that have the ability to incorporate into the genome but can survive out of chromosomes, with or without replication, and are kept within plasmids for further reproduction. Genes of genetic parasites such as viruses, plasmids and transposons have a significantly higher ability to be successfully transferred into the cell genome. In such a manner they function as vectors for horizontal gene transfer regulated by inner characteristics of the organism, linked to the specific ecological conditions (Dale 2002; Snow *et al*. 2004).

Evidence of horizontal transgenic DNA transfer

It is believed that once incorporated transgenic DNA becomes stable. However, there is a body of evidence against this assumption. There are molecular data that prove structural stability of transgenic DNA, with regard to its location, point of penetration into the genome and gene arrangement within the following generations. Transgenes can be either stabilized in successive generations or completely lost. Herbicide tolerance gene incorporated into *Arabidopsis* with a vector can show up to 30 times stronger vector avoidance behavior, and it spreads as the identical gene obtained by mutagenesis. The results obtained suggest that horizontal gene transfer can occur via insects visiting plants for pollen and nectar, and that pollen can transfer transgenic DNA into the bee larvae gut (Thompson et al. 2003; Snow et al. 2004).

Secondary horizontal transfer of transgenes and antibiotic resistance marker genes from genetically modified plants into soil bacteria and fungi has been experimentally confirmed. The transfer to fungi was achieved by simple co-cultivation, while the transfer to bacteria by reisolation of transgene DNA or a whole DNA of a transgenic plant. Successful transfer of kanamycin resistance marker genes into the soil bacterium *Acinetobacter* was achieved using total DNA extracted from homogenized plant leaf of the following transgenic plants: *Solanum tuberozum* (potato), *Nicotiana tabacum* (tobacco), *Beta vulgaris* (sugar beet), *Brasica napus* (canola) and *Lyopersicum esculentum* (tomato). It is estimated that about 2500 copies of kanamycin resistance genes are sufficient for a successful transformation of one bacterium, regardless of the fact that there exist 6 millions of folded strands of plant DNA.

However, the natural conditions in the environment are widely unpredictable and some studies on synergistic effects cannot be neglected in this case. Free transgenic DNA would be free in the rhizosphere around the plant roots, which is a significant critical point in the environment. Some other scientists have found horizontal transfer of kanamycin resistance gene from transgenic DNA to *Acinobacter*, and positive results have been obtained by the use of homogenized 100μ of plant leaf.

The biotechnology industry insists that the existence of horizontal gene flow in laboratory conditions does not mean that it occurs in natural environments. However, there is a body of evidence showing that that it can and does occur in nature. Above all, genetic material taken from dead and living cells resists external conditions, does not dissolve or devastate as previously assumed. This indicates that sand, humus acid particles and plant debris enable infections with more microorganisms in the soil. Bacterial transformation in the soil via DNA absorbed into sandy clay has been confirmed by various experiments on microorganisms.

In 1993 researchers in Germany started a series of experiments with rhyzomania resistant transgenic sugar beat plants (Pidgeon et al. 2001), containing the marker gene for kanamycin resistance, to study the stability of transgenic DNA and horizontal gene flow of transgenic DNA into soil bacteria. Thus, horizontal gene flow is a leading phenomenon that has occupied significant place in the evolution of species, and it still does. All this suggests that natural horizontal gene flow is a regulated process, limited by specific obstacles and mechanisms of rejection and inactivation of foreign genetic material. Genetic engineering has created great variability of artificial construction designed to overcome barriers between all kinds of organisms and forestall all genomes (Robinson et al. 2000; Glišin 2005; Topisirović 2005).

Stress tolerance and plant resistance to herbicides (HRC)

Great efforts have also been made to increase crop tolerance toward herbicides, and it has been tested worldwide (Kwon et al. 2001). Herbicide tolerance can be achieved through an increase in protective mechanism, by reduction of herbicide uptake, by degradation or reduction of susceptibility (Caseley et al. 1991; Bradley et al. 2000, Konstantinović and Bošković 2001). Herbicide tolerance genes are widely used as markers in transgene plant selection. If genes having different tolerance to herbicides are developed or incorporated into identical plant species, they may stipulate the creation of weeds with multiple resistance genes (Colbach et al. 2001a; 2001b; Dewar et al. 2000; 2003; Desplanque et al. 2002; Richter 2002a; 2002b).

Numerous genes involved in tolerance to various environmental stresses were inserted into plants. Greenhouse experiments showed that metallothioneins inserted in tobacco increased cadmium tolerance. Manipulating genes that affect compatible solutions, such as betaine-aldehyde dehydrogenase, improves tolerance to salinity. The spread of crops tolerant to drought and salinity could increase the range of wild relatives, making them more competitive, with consequential negative effects on ecosystems and agroecosystems (Beringer 2000; Fagan et al. 2001; Marshall 2001).

Hybridization of herbicide resistant crops (HRC) with populations of wild relatives makes these plants complicated for control, especially if they possess resistance to widely used herbicides (Altieri 2000, FAO 2003; Firban et al. 2003). Transgene plant resistance to herbicides (Kwon and Kim 2001; Lutman and Berry 2003) makes chemical control easier, above all because it includes compounds that are active to a very wide spectrum of weed species (Kim 2001; Kudsk and Streibig 2003).

Quality characteristics of GM plants

Transgene diversity is directed towards the improvement of products derived from cultivated plants. The effects of these changes on the whole plants are reflected in their metabolism and are of immediate interest to their commercial application. Some of these plants, especially commercially produced "antisense" tomatoes in the United States have been tested in field trials. The simplest change is probably the removal of the natural gene, for example," antisense " tomato polygalacturonase gene.

Other modifications include metabolic pathway changes via the introduction of various metabolic enzymes from other species, turning the natural path to a different end product. This can result in manufacturing plant products in different plant species, animal or bacterial products in plants or completely new products (Robinson et al. 2000, FAO 2003). There are no a priori concerns about these types of GM plants, because the modifications are very different. Metabolism modification in conventional selection sometimes produces unexpected and undesired secondary effects. Unacceptably high levels of tannin were found in brown sorghum seed cultivated for resistance to birds.

Therefore, a random change of characteristics, such as the nutrient value of main product or an increase in toxic secondary metabolites, should be carefully considered. It is necessary to collect as many biochemical and other data on transgenic plants as possible before they are widely accepted, in order to avoid unintended consequences that may become a problem (Gueritaine et al. 2003). Adaptive effects of plants or hybrids with new characteristics must be studied case by case (Tester 2001, Conner et al. 2003).

Non-cultivated populations of transgenic plants

The possibility of introduction of transgenic plants will be highly dependent upon their capability to adapt to the new environment. For many crops it is known that they form temporary non-cultivated populations, and these are species such as canola, lucerne, radish, carrot, rye, clover, sugar beet, chicory, beet, cabbage, of which some are native, whereas the others have probably been introduced for cultivation (Marlander et al. 2003). In some cultivated crops, i.e. rye, difference between non-cultivated and natural population is unclear, whereas in case of some other species the colonization is not extensive and there probably has been no harmful influence on non-cultivated plant species. Possibilities of gene flow from trial fields under GM plants through pollen will depend upon sexual compatibility between GM crops and their wild relatives, and possibilities for pollination and seed obtainment (Pretty 2001).

The frequency of occurrence of this gene transfer will be influenced by the important spatial isolation between GM crops and a suitable recipient, depending upon method of pollination, wind or insects, isolation in time, i.e. flowering season.

The experiments have been carried out in order to determine rates of cross-pollination between GM and non-GM potato planted at different spatial distances one from another (Hudson et al. 2001). The results were well harmonized, both showing that transgene movement outside the GM trial field has been negligible at distances less than 10 m, and low rates of cross pollination that is usually present in potato have also been in accordance.

In contrast, in canola seed, compatible inbreeding is present, it can produce huge seed quantities, it is pollinated by wind and insects. Pollination at huge distances happens probably due to insects; airborne pollen can be found 30-50 m away of canola plants, but it is reduced with increasing distance (Bartsch et al. 1999). Darmency et al. (1998) have estimated the importance of pollination performed by honeybees in pollen dispersal from transgenic oilseed rape and concluded that although bees can travel up to 1-2 km from their hive, the food is carried from the vicinity of the hive.

Experimental trials on fields that use GM or non-GM plants can provide useful data in regard to necessary isolation distances used to avoid the release of transgenes. However, trials on natural populations suggest that the situation could be more complex, and sub-classifications of local populations can strongly influence transgene incorporation into wild populations (Dale 2002).

Interpretation of the results has also been complex, and Breeze et al. (1999) emphasize the prevailing importance of the calculation of dose changes in relation to distance from a GM experimental field, over the absolute percentage of GM seeds at a given distance from the plot. However, gene flow depends not only on crops, but also on variety, location and season (Marshall et al. 2003). Experiments on gene flow in wild radish populations suggest that the size of donor and recipient populations plays a significant role in gene flow. Thus, huge pollen sources, such as a great spectrum of GM gene introduction, have significant influence on small wild populations of compatible plants. They also note that there are noticeable variations in gene flow evaluations, probably due to local-positional and pollinator effects. Other researchers have found similar effects in *Cucurbita* (Moonen and Marshall 2001) and rice (Song et al. 2002). Further work on the spread of genes in populations can be necessary for assessment of potential transgene dispersal. Such assessments become particularly important in analyzing the possibilities of transgene transfer from cultivated crops to related species (Darmency et al. 1998; Rongli et al. 2000).

Gene transfer from crops to weeds

The Global Wheat Group has identified three crops that have sexually compatible weed relatives that will probably be subject to gene transfer in agricultural systems.

Breeding via pollination and production of fertile hybrids varies from case to case. Even when chosen characteristics have positive advantages, introgression of new characteristics into the existing weed population is still possible

(Konstantinović and Bošković Jelena 2001). Risk of environmental damage, then, depends upon the weed habitat. In weed-crop complexes (Hauser and Ostergård 1998) analyzed in these studies, where the habitats of weed relatives are restricted to agricultural systems, it is not possible for a new trait to endanger natural ecosystems (Creswell et al. 2002).

Role of agricultural weed ecology in accidental hybridization

Weed ecology and evolutionary biology are of high importance in assessing the prospects of accidental flow of resistant transgenes to harmful organisms in agricultural weed populations (Bradley et al. 2000; Marshall et al. 2003).

The accidental transgene flow model has three phases that lead to creating widely distributed transgene carrying weed populations. The first phase is hybridization between the weed and the transgenic crop. The second phase is the occurrence of introgression and adaptation processes with evolutionary mechanisms that improve unadaptable traits in the hybrid products of earlier generation. As a consequence, weeds transfer resistance transgenes to harmful organisms (Buckelew et al. 2000) and, hence, have high adaptation level for certain agro ecosystems. Finally, the process of dispersion and dissemination of these new weeds in nature, together with local adaptation of various conditions, is important during transition of a sufficiently wide area.

Weed ecology and evolutionary biology are important for understanding the interaction between the three phases. The actual scientific data on weed ecology are still lacking. During the last few decades, scientists have focused on herbicide weed control. Amongst these prevailing studies, ecological research and especially theoretical description have been neglected (Altieri 2000; Konstantinović and Bošković 2001; Conner et al. 2003). Hybridization between transgenic or conventional plant species and sexually compatible relatives occurs in many crops and produces new forms of weeds in newly obtained populations. Numerous papers have provided a detailed description of this hybridization and it can be expected that transgenes will even transfer across large spatial scales and across a range of genetic incompatibilities (Perry 2002). In some systems, the accidental transfer of transgenes by hybridization seems unavoidable. However, in other cases it is not clear if hybridization is a proportionally limiting phase in transgene transfer. It is assumed that hybridization can even be proportionally limiting in some circumstances, i.e. when occurring across a range of incompatibilities. Weed ecology aspects that can affect hybridization levels in these situations include weed cropping systems and effects of spatial and temporal distribution of weeds in several phases.

Weed selection system in field agro-ecosystems of crops is a mixed system of fertilization involving inbreeding and cross fertilization (outbreeding), although other reproductive systems have also been known. Therefore, the widely

distributed weed selection systems enable hybridization, but such fertilizations must happen during significant levels of inbreeding.

For selection systems and other aspects of genetic systems and reproductive ecology, the influence on hybridization levels is known to vary within and between weed populations. For instance, Datura stramonium populations in Northern Carolina have flowers that open to pollinators and exhibit approximately 10% outbreeding level. Quite the contrary, certain populations are exclusively self pollinating, with flowers that do not open to pollinators. In some cases this variation refers to the adaptation of the selection system after great expansion (Cresswell et al. 2002). However, the attitude to pollinators can vary in many ways, even geographically. These aspects of reproduction therefore should not be considered as permanent characteristics within weed species (Pidgeon et al. 2001; Moyes et al. 2002).

Spatial weed distribution can strongly influence the hybridization of weed – plant species. First, many weeds are unevenly distributed in fields. As described in some papers, uneven distribution in some species has a certain level of temporary stability (Perry et al. 2003). Uneven distribution can be caused by edaphic factors or by persistent effects of high seed production. Within a field, uneven weed distribution can reduce weed-crop hybridization. Weed occurrence in plant populations at an adequate density, with a small proportion of single weeds at the ends of these plant populations, limits the plant populations proportionally to hybridization. More homogeneous and uneven distribution can significantly favor considerably higher levels of cross fertilization. Locally isolated individuals can have higher quantities of crop pollen due to changes in the movement of pollinators as a function of local density. Weed density can have a countereffect on hybridization levels, when plant serves as the female parent. In this case, high densities can favor hybridization due to the advantage of achieving high local weed pollen densities, and homogenous weed density can reduce plant hybridization. Weed distribution in a wide area around field crop agro-systems also show potential importance in determining the level of weed-crop hybridization. If conditions allow weed stabilization in areas that are not under crops in the region, many small, isolated populations can exist (Pierre et al. 2003).

Weed-crop hybridization can occur at higher levels in these populations than in populations in the field due to a number of reasons. For example, due to differences in reproduction in many weed species, flowering can occur over a long period of time during the growing season of a given species. Seeds from commercial crop fields, pollinated by wind or insects, scatter for more than 1 km outside the field. Therefore, the crop pollen can be expected to reach weed populations outside the agricultural fields within this distance. When considering weed populations in a particular environment, the proportion of intense flowering

and the presence of pollen of cultivated plants can significantly extend the range of opportunities for hybridization in many weed-crop systems.

Many weeds are highly variable. During certain years weather factors can lead to failure in controlling weeds in the wider region, resulting in a high density of weeds in some areas. Weed density varies on a regional basis due to the interaction between the biology of weeds and regionally dependent different ways of weed distribution and other factors of the breeding system. Both forms of variation can result in a significant increase in the absolute number of hybridization cases.

The level of hybridization is affected by weed density, dependent variations in pollinator behavior or spatial distribution. For example, an increased local presence of the species may enable them to settle border habitats in agricultural areas, which are densely populated, probably increasing the likelihood of hybridization. Therefore, levels of hybridization can vary significantly over the years and a number of weed species (Rongnli et al. 2000, Richter et al. 2002a, 2002b).

Transgene introgression and weed adaptation

The evolutionary process that follows hybridization will certainly be influenced by many ecological weed properties in agrosystems of cultivated plants (Kwon et al. 2001). The nature of these systems seems prevalent only for strongly expressed factors of weed population regulation (Pidgeon et al. 2001), compared with the majority of annual plant populations, which are short-lived and which inhabit other types of ecosystems.

This can facilitate transgene introgression even if the hybrids and the starting backcrossed generation have a low level of adaptive features in comparison to weeds that do not carry transgenes (Fagan et al. 2003; Kim 2001).

Weed populations are frequently small and sometimes temporary; therefore, the effects of selection, migration and random genetic changes will influence the evolution of introgression. Seed ecology is of primary importance for weed survival. Accordingly, the effects of transgenes on other genes in plant species and seed ecology will probably induce strong selective effects in these genes. Introgression of genes that improve adaptation of weeds to these predominant selective factors can significantly increase the average adaptability of weed population. Exchanges between adaptations to different limiting factors that result from the introgression of a single gene can also be minimal. The best example is the evolution of herbicide resistance in weeds (Kwon and Kim 2001; Lutman and Berry 2003; Pierre et al. 2003).

The occurrence of herbicide resistance often significantly increases the average survival and population growth of weeds. Herbicide resistant mutation can have high absolute adaptability, despite the basic functional damages caused by pleiotrophic effects of resistance mutations. This shows how selection can favor a mutant that exceeds limiting factors. The other line of evidence rises from

multiple examples of increased distribution and density of weeds from field trials over hybridization (Perry et al. 2003). Finally, many cases of the basic increase in distribution and density of certain weeds follow moderate changes in cropping systems, providing the additional proof that many weed communities are regulated with several strong factors (Rognli et al. 2000).

If correct, this assumption suggests that the adaptation of weeds can rely upon randomly transferred transgenes after hybridization and is facilitated by the biological uniformity of the actual field crop ecosystems. Weeds can demand relatively narrow evolutionary movement, given the break of linkages toward undesirable characteristics of plant species in accordance with adaptation to a wide area (Saeglitz et al. 2000). One criterion for the assessment of the transgene spread into weed population is that the survival rate of the hybrid weed-crop that carries transgene should be higher than the adaptability of non-hybrid weeds. This criterion can be much easier met in temporary ecosystems of field crops than in the majority of others. Therefore, the transfer of transgenes can be a fast process. Even hybrids with very low adaptability and early back-crossings can survive in agrosystems in adequate densities. There is a possibility for introgression and adaptation that make their survival easier. These assumptions can be applied to accidental transgene transfer that affects tolerance to abiotic factors. However, weed populations in wider areas can be restricted by one biotic factor, adaptation to which would provide basic survival increase (Streinbrecher 1996; Snow et al. 2004).

The other characteristics of weed ecology that probably affect adaptation of the crop-weed hybrid is the frequency of occurrence of low efficient population size and high levels of self-fertilization, especially during the colonization process (Colbach et al. 2001a; 2001b). Small population sizes cause random changes in the genetic composition. These mechanisms can act at genetic base produced by hybridization, producing a number of genetically differentiated small populations from genetically different back-crossed weed populations.

This implies that adaptation in weed populations that contain random transgenes will probably be influenced by selection and random genetic change. The presence of both factors enables the occurrence of evolutionary processes that do not occur when selection is the dominant evolutionary mechanism. Specifically, adaptive effects of transgene combinations, other crop genes and weed genes can be more adaptable in expression with the mutual action of random genetic exchanges in regard to the selection that acts alone (Conner et al. 2003). These mechanisms can be especially expressed when weed populations have high levels of weakening and repeated colonization, forming ecological and genetic metapopulations. Although it is still not clear if weeds in agriculture have metapopulative structure, the occurrence of such structures, in combination with small sized population and altering selection pressures, creates convenient conditions for equilibrium processes. However, the effect of these processes can itself be unpredictable due to geographic variations in population structure in some weed species due to the cropping system, local adaptation after colonization, time span from colonization and hybridization with related taxonomic categories (Dale 2002; Marshall and Moonen 2002).

The final dimension of weed ecology in relation to adaptation after hybridization is the ecology of seeds. The ability to maintain seed stability in the soil, together with effective spreading and the ability to reproduce quickly and efficiently are the most important features in the spread of weeds in crop field agro-ecosystems (May 2003). The weed populations dynamics has shown that seed demography (i.e. survival and germination levels) greatly affects growth levels of weed populations. The longevity of weed seeds varies considerably between species. Many agricultural activities affect the demographics of seed amounts in the soil, preventing germination or otherwise increasing the seed extinction levels. These factors include stubble crops, tillage and residue burning, that can affect the seed directly or indirectly, via effects on the animals that feed on seeds and pathogens. Germination and dormancy of weed seeds is important for the survival in any given developmental system (Marshall 2002).

If the transgenes affect the weed ecology, the resulting effects are likely to act as factors dominant for the survival (Sharma et al. 2000) hence leading to weed infestation. Similarly, the unadjusted effects on seed ecology may be the primary mechanism by which non-transgenic crops reduce the adaptation after hybridization. There are several other aspects of weed seed ecology affecting the adaptation phase. The first is the well-known effect of dormancy, where the genotypes of plant weeds that grow outside environmental conditions can again be separated for storage during periods of adverse conditions. Therefore, seed populations are affected by the genetic variability of weed populations. Also, seed population, as a form of temporary expansion of genotypes, allows weeds to be tested over a wider range of conditions, which would not be possible otherwise. This effect can significantly increase the possibility for a weed carrying accidentally transposed transgene to appear in an environment to which it was adapted. Molecular and biochemical data on homology levels between species of crops and their wild relatives worldwide indicate gene introgression from crops into populations of wild relatives (Nakayama and Yamaguchi 2002), in various plant species including maize, melon, carrot, sugar beet and rice. The possibility of transgene introgression into wild populations over time within some species and in some geographic areas would be very high. Hybrids between crops and their wild relatives most probably occur in crops with low adaptation grown in their region of origin. Minimal divergence and maximum exposure from the other relatives should be provided. The level of transgene introgression from crops into wild species is influenced by factors such as overlapping of flowering period with wild species, and the ability of hybrids to back-cross with wild relatives within the population (Darmency et al. 1998). This suggests that the possibility of gene transfer is especially significant in tropic areas, since many important crop species evolutionarily originate from these areas.

Isolation distance or incompatible flowering time can be realized through the use of lines - barriers with different species, guard-lines with the same species

and application of genetic engineering causing male sterility (Saeglitz et al. 2000) that might contribute to genetic isolation. Evidence suggests that isolation itself could reduce the degree of introgression due to pollinating insects (Sharma et al. 2000, Bouchard et al. 2003), while the use of barriers or guard plants could be useful for limiting the spread of GM pollen from experimental fields. Other proposed methods of genetic isolation include selection for increased feeding in GM crops or reduced sexual compatibility with wild relatives. The use of suicide genes was also considered, however, such measures involve significant limitations in the application of GM plants.

Spatial seed dispersion and weed colonization

Efficient spatial dispersion of seed is considered a primary feature of weeds (Kudsk and Streibig 2003) and weed ecology is expected to influence the fate of the accidentally transferred spread transgenes in a number of ways. On a field scale, simulation modeling indicates that high levels of weed seed dispersal generally greatly increase the population of weeds (Pidgeon et al. 2001). For most weeds in field crops, dispersion is determined by the interaction between weed characteristics and human activities, such as contaminated seeds of cultivated plants, equipment, water for irrigation and seed transmission. When human activities become the main vectors of the spread of weed seed, those dispersions are difficult to characterize, due to the geographical variations of the processing system. As a result, the maximum spreading distances are not known in most cases.

On a wider scale, many cases of rapid expansion of sub-continental weed species are known. Weed species were found to become abundant over large areas in the west of the USA (Rognli et al. 2000), due to the changes in farming methods that improve their abundance, such as Aegilops cylindrica, a sexually compatible weed in wheat. Weeds resistant to herbicides spread over hundreds of kilometers of road shoulders in less than a decade. These observations suggest that weed populations of road shoulders of other non-field habitats may be important for the spatial spread of weeds, recognizing the importance of weed ecology in agricultural areas and the accidental spread of the transgenes (Altieri 2000; Marshall 2002). Herbicide resistant crops can be agronomically harmful if a resistant species germinates before the sown plants germinate, or if a herbicide resistant plant occurs as a weed on another field (Tyystjarvi 2009).

Weeds can have significantly higher adaptability over large spatial areas of the transferred transgene. In theory, the resulting spatial homogeneity of a suitable habitat (Perry 2002; Conner et al. 2003) and the absence of the need for local adaptation accelerate the rapid expansion of the colonizing organisms. Therefore, the ecology of weed spread and population regulation in agro-

ecosystems and agricultural areas seem to enable large and rapid expansion of the adapted weeds.

The properties of weeds that affect their spread (e.g. seed size, shape, similarity to crop seeds, etc.) should be considered adaptive characteristics that are probably the result of strong selection. The effects of crop genes and ecological expansion can adversely affect the adaptation of hybrids. The spread of weeds may have an evolutionary role. In small basic populations it can cause an adaptive process, which does not occur in large populations. In the increasing balancing process, the spread of weeds has an important role in evolution, moving into small populations in other areas and affecting changes in other populations.

The weed ecology in cultivation systems can facilitate random transgene transition, allowing the survival of weed-crop hybrids that are not adjusted, compared to wild-type weeds, in a series of adaptability components. This probably happens when the hybrids and next generation back cross, carrying the appropriate transgene adaptive value. The ecology of seed, the expansion of some basic characteristics responsible for weed adaptability and the level of population growth are not widely accepted parameters. Effects arising from transgenic and other crop genes will greatly influence the adaptation of weed-crop hybrids and back cross generations. Most of the main weed species show intensive spatial and temporal variations in reproduction, ecology and seed spread on several scales. This variation has both genetic and environmental causes (FAO 2003; Snow et al. 2004). The populations of agricultural weeds are widespread in agricultural regions, including many populations that appear outside the farmland. The specific ecology of these populations may affect all phases of the random transgene transfer (Dewar et al. 2003).

The influence of GM plants on biodiversity

One of the limitations for the introduction of GM plants into the environment is the concern about their adverse effects on biodiversity, including the possibility of its devastation. Fear of loss of biodiversity (Anon 1994) is an important basis for the opposition to genetic modification and GM plants by several influential environmental protection research groups from around the world.

The influence of GM plants on biodiversity is a complex complicated problem. Scientific studies and discussions have been directed towards the understanding whether GM crops have an impact on biodiversity and defining their qualitative and quantitative differences from commercial crops. Biodiversity and agriculture are strongly interrelated since biodiversity is crucial for agriculture, while agriculture can contribute to sustainable use of biodiversity (De Jaramillo 2009). Biodiversity is very important for the survival and

maintenance of global planetary conditions, providing the aesthetic, scientific, cultural and other values. The general value of the world's biodiversity is estimated at about 33 trillion $ per year (Costanza et al. 1997; Boskovic et al. 2012a). As regards the multidimensional complexity of the biodiversity concept and considering the significance of the technological development of GM plants, further studies that will clarify this interdependence are needed. In a broader sense, they will rely upon the social, economic and political context of genetic modification applications that will determine the risks or potential advantages of GM plants to biodiversity.

Transgenes

Genetic modification itself does not substantially change anything, but the adaptability of GM plants will depend on transgene effects and the impact of a particular stage in transgene development. For example, the length of seed vitality and seedling stabilization can be an especially important modification of oil crops seed. Both may be more important for the stabilization of annual plants, in relation to the characteristics that affect the survival of adult plants or fertility. The effects of transgenes within natural populations of ecologically important plants, which may have a greater effect on the species carrying the transgene, were also examined (Song et al. 2002; Boskovic et al. b). If transgenes were giving selective advantage to a wild type, it might become dominant, which would result in a reduction in the natural variation (Steinbrecher 1996).

Certain characteristics of transgenic genetic modification may present an advantage in some environments, e.g., tolerance to salinity, drought, cold and pest resistance. A wide variety of existing genes for transgene transfer (Watkinson et al. 2000) and the changes feasible by molecular techniques make this technology fundamentally different from traditional methods of selection.

Monitoring

Ecological monitoring of GM crops in complex ecosystems is needed even after commercialization. This complexity varies from year to year and indicates indirect biotic effects. As laboratory and field experiments cannot sufficiently repeat all interactions that occur in one ecosystem, the only way for evaluation of the full level of ecological effects of GM plants is monitoring in natural ecosystems. As some of these effects cannot be predicted in advance, ecological monitoring will be needed to reveal and differentiate between existing ecological influences (Altieri 2000; Dale 2002, Bošković and Isajev 2007).

Environmental monitoring is very expensive; therefore, the information obtained should be used within the clear adaptive management system. Such management includes repeated cycles of firmly set rules within specially designed programs, the use, evaluation and estimation of the monitoring as a whole (Tester 2001; Snow et al. 2004).

A more significant problem in GM plant monitoring is the lack of adaptive management systems specifically developed for this application. To control the evolution of resistance to pathogens and pests, the frequency of resistance should be monitored in the field, with additional research conducted in order to set standards for monitoring and determining appropriate management that can clarify the problem (Boskovic et al. 2010b). Monitoring of new GM plants will need to be evaluated by a diverse group of scientists and scientific disciplines including agriculture, forestry, ecology of wetlands, entomology, pathology, etc. In the future, scientists and technological advances will continue to expand opportunities for artificial design and construction of plant organisms. Genomics and bioinformatics will facilitate identification of commercially important genes that can potentially be transmitted between species. Environmentalists will more significantly contribute to the wider debate on the public contribution to the prevention of risks associated with those innovations.

Conclusion

Biotechnology alongside the introduction of genetically modified (GM) crops is constantly providing new opportunities for increasing crop productivity and tackling problems in agriculture, such as diseases, pests and weeds, abiotic stress and nutritional limitations of staple food crops. Crops possessing new traits enabling their use in pharmaceutical production are also being generated. As GM crops are being introduced into various locations characterized by different ecosystems, agriculture, biodiversity and agriculture practices, a scientifically based understanding of the environmental effects of GM crop cultivation would assist decision makers worldwide in ensuring environmental safety and sustainability. The main important environmental assessment of GM crops deals with their putative invasiveness, vertical and/or horizontal gene flow, effects on biodiversity and the impact on other products. These investigations are all highly interdisciplinary and complex. This paper deals with some of the most important problems related to the introduction of GM crops into the environment, such as plant protection, ecological effects of HRCs, gene flow, biodiversity, stress, ecological risks of Bt crops, effects on soil ecosystems etc. There is a clear need to further assess the severity, magnitude and scope of risks associated with the massive field deployment of transgenic crops. When assessing GMC interrelation with the existing cultivars, an increased knowledge base underpinning the development of GMC will provide greater confidence in plant science while assessing the risks and benefits of releasing such crops.

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OCENJIVANJE EKOLOŠKIH ASPEKATA BIOLOŠKE SIGURNOSTI GENETSKI MODIFIKOVANIH BILJAKA PO ŽIVOTNU SREDINU

- pregledni rad –

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Rezime

Biotehnologija uz uvođenje genetski modifikovanih (GM) biljaka konstantno stvara nove mogućnosti povećanja biljne proizvodnje i reševanja problema u poljoprivredi, kao što su bolesti, štetočine, korovi, abiotički stres i nutritivna ograničenja. Stvaraju se i biljke koje poseduju nove osobine, koje omogućavaju njihovo korištenje u farmaceutskoj proizvodnji. Budući da se GM biljke uvode na različite lokacije okarakterisane raznovrsnim ekosistemima, tipovima poljoprivrede, biodiverzitetom i agrikulturalnom praksom, neophodno je naučno razumevanje efekata uzgoja GM biljaka na životnu sredinu, koje će obezbediti bezbednost i održivost životne sredine. Najvažnija istraživanja su ona usmerena na invazivnost GM biljaka, vertikalni i horizontalni prenos gena, uticaj na biološku raznovrsnost i na druge proizvode. Ova ispitivanja su veoma složena i multidisciplinarna. Ovaj rad razmatra neke od najvažnijih problema vezanih za uvođenje GM biljaka u životnu sredinu, kao što su zaštita biljaka, ekološki efekti horizontalnog prenosa gena, biodiverzitet, stres, efekti na zemljište itd. Postoji jasna potreba da se dublje istraži veličina i opseg rizika povezanih sa masivnom upotrebom transgenih biljaka. Pri procenjivanju međuodnosa GM biljaka i postojećih

sorti, detaljnije poznavanje razvoja GM biljaka će omogućiti jasnije, pouzdanije i preciznije usmerene aktivnosti u biljnim naukama.

Ključne reči: genetski modofikovane (GM) biljke, životna sredina, zaštita bilja, horizontalni prenos gena, biodiverzitet