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Preliminary Risk Assessment of Transgenic Plant Use in Ukraine

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Proposed research for Ukraine is an attempt to adopt and develop a system of pre-release risk assessment of GM crops after the idea of F.T. de Vries and developed by K. Ammann based on integrated information (specific codes) on hybridization potential, dispersal of diaspores, and frequency of distribution of respective species. After an evaluation of all codes, the combination gives an opportunity to estimate the impact of GM plants on wild flora. Six categories of risk probability have been developed from 'no effect' to 'substantial and widespread effect.' Accordingly, conditions of field releases are defined to the risk category term of monitoring and level of containment of GM species belonging to these categories. In the case of Ukraine, such preliminary risk assessment was done for oilseed rape, sugar beet, potato, corn, and false flax. The proposed technique can be used in the risk assessment of plant introduction. In addition, it is advisable to apply it for novel plant varieties developed using new breeding techniques.

***Key words*:** sugar beet, corn, false flax, oilseed rape, potato, preliminary risk assessment, transgenic plants.

Any attempt to create new crops or improve an existing crop is accompanied by certain environmental impacts. In the last 15 years, significant public attention has been focused on the problems of large-scale cultivation of transgenic or genetically modified (GM) crops. The use of such crops can solve a number of acute problems in agriculture. At the same time, the media has published articles about the negative effects of GM organisms on human health and the environment. Taking into account the cautious and often openly negative attitude of society toward biotechnological varieties, public interest in the environmental and food safety of novel products is always present. Official bodies often oblige by providing numerous, cumbersome, and not scientifically substantiated checks. This process unnecessarily hinders the introduction of newly developed varieties. Therefore, it is important to create a universal system of science-based risk assessment of GM plants; this would be optimal in terms of time and cost and provides the necessary level of safety.

One of the most important aspects of this problem is to assess the risk of vertical gene flow from transgenic plants to the wild populations of sexually compatible species. Although there were no scientifically confirmed cases of negative effects on the environment caused by vertical gene flow, we cannot rule it out in the future. Risk assessment requires a generalization of the many factors that affect vertical gene flow in complex biological and ecological systems. First, the risk assessment should take into account the geographical conditions, biological behavior of a transgenic species, and their wild relatives growing in respective geographical zones (i.e., in Ukraine). Therefore, this study looks at the preliminary risk assessment of GM plants before their field release in Ukraine.

Scientifically grounded risk assessment requires a multidisciplinary approach in order to make decisions that would be understandable and acceptable to all. For those purposes, we adopted usage of the criteria based on calculation of gene flow indices (Novozhylov & Blume, 2001) after the idea first proposed by F.T. de Vries (de Vries, 1996; de Vries, Van der Meijden, & Brandenburg, 1992) and further developed by K. Ammann for Switzerland (Ammann, Jacot, & Rufener Al Mazyad, 1996). Respectively, we summarize main results about adaptation of the above mentioned system for potential risk evaluation of possible spread of genes from dedicated transgenic crops to wild flora of Ukraine.

A significant problem for biodiversity lies in the introduction of new plants held throughout the world. We identified dozens of cases of conversion of plants introduced (planned or accidentally) in new geographical conditions in aggressive weeds. Moreover, until now nobody has assessed the risk of new plants obtained through conventional breeding methods (mutagenesis, hybridization). But at the same time, new plant forms may also appear to be undesirable for wildlife features. The proposed method can be successfully applied to the evaluation of the risk of plant introduction, and to plant varieties bred by new "conventional" techniques.

Criteria for Preliminary Risk Assessment of Transgenic Plant Use in Ukraine

To address this issue, the codes proposed earlier (Ammann et al., 1996; de Vries, 1996; de Vries et al., 1992) were used to make preliminary rough estimates of the potential risk stemming from the introduction of GM sugar beet, corn, oilseed rape, potato, and false flax in Ukraine. Following this, more detailed risk assessment based on field monitoring and experimental approach—where judged necessary—were undertaken. Classification of these codes includes

1. codes of dispersal of pollen (Dp),
2. codes for dispersal of diaspores (Dd), and
3. codes for frequency of distribution (Df).

This classification covers the areas of dispersal of pollen and hybridization potential, dispersal of diasporas, and frequency of distribution. Within each category, indexes range from 0 (lowest risk) to 5 (highest risk) and U (unknown). Below is a description of these codes in subsequent order.

Dp: Hybridization and Pollen Dispersal Index

The Dp code encompasses hybridization potential and the spread of pollen, partly including the possible negative effects of the transgene.

Dp 0 Code. The plants that belong to this category have no wild relatives in Ukraine, so there is no chance for hybridization and no environmental effects. Field release of such plants is possible without preliminary experiments.

Dp 1 Code. The plants in Ukraine in this category have no sexually compatible relatives; it has been experimentally proved that hybridization between wild and cultivated plants is impossible. In this case, there is also no chance of hybridization and no environmental effects. However, this case should hold experiments to study the impact on the ecosystem when the transgenesis provides new sustainability or competitiveness.

Dp 2 Code. The natural hybridization is possible in the experimental conditions and offspring is fertile, but not registered spontaneous hybrids. They have little chance for gene transfer, but under certain local or artificial conditions in agricultural systems, it may be implied as a possible rare phenomenon.

Dp 3 Code. There is occasional natural hybridization, but no backcrosses have been observed in Ukraine. The local conditions must be thoroughly examined. This should be applied using a case-by-case approach from species to species and from region to region because possible genes flow through pollen in certain groups of species in unfortunate circumstances may occur. In some cases, hybrids may tend to create local invasions.

Dp 4 Code. There is natural hybridization, and hybrids are fertile and form a backcross. There is a chance of natural hybridization and are quite often observed back crossing. Sometimes small natural fertile hybrid populations arise. Should be applied using a case-by-case approach from species to species, region to region, and step by step.

Dp 5 Code. Natural hybridization occurs fairly often; hybrids are fertile and backcross frequently. Vertical gene flow occurs very frequently, and hybrid populations are often detected in nature. And in this case, it should be applied using a case-by-case approach from species to species, region to region, and step by step.

DpU. Data are too scanty or lacking, so no evaluation is possible.

Dd: Diaspore Dispersal Index

Dd 0 Code. No chance for diaspore dispersal (seeds are sterile or deficient and have lost their reproductive function). Field release of such plants is possible without preliminary experiments.

Dd 1 Code. A single diaspore dispersal is possible in extremely successful conditions. Plants usually survive only for one season since they are not adapted to our climate and relatively Ukrainian ecosystems are not expected to endure any environmental effects.

Dd 2 Code. Diaspore dispersal slightly—but under favorable conditions—is possible. It is necessary to further study the population dynamics.

Dd 3 Code. Chances of diaspore dispersal are real. Fruiting of cultivated plants is not desirable and should be eliminated by various methods. It is necessary to further study the population dynamics.

Dd 4 Code. The chances of the diaspore dispersal in the nature are real. Fruiting of cultivated plants is normal during cultivation. It is also necessary to further study the population dynamics.

Dd 5 Code. Diaspore dispersal in wild nature is the rule. Fruiting occurs frequently and is abundant. It is also necessary to further study the population dynamics.

DpU. Data are too scanty or lacking, so no evaluation is possible.

Df: Dispersal Frequency

Df 0 Code. This code includes wild relatives or species not known in the wild or feral populations in Ukraine. They have no environmental effects from the introduction of such plants. Field release of such plants is possible without preliminary experiments.

Df 1 Code. Wild relatives extremely rare in the wild and that do not occur as feral populations in Ukraine have this code. There is practically no chance for hybridization.

Df 2 Code. Wild relatives very rare in the wild and/or they occur sporadically as feral populations in Ukraine are in this group. Their distribution is difficult to predict because it is essentially out of control. The chances of hybridization are paltry, but unpredictable. Certain environmental effects can be expected, but in most cases they are on a small scale. For the cultivation of such plants, one must choose a place to avoid hybridization or any ecological effect.

Df 3 Code. Wild relatives and/or their feral populations not very common in the wild in Ukraine still occupy a stable place in the ecosystem. Wild or feral populations are known in Ukraine, but not common. The chances of hybridization with wild populations exist, though only marginally. Some effect of the introduction of crop plants can be expected if they and their wild relatives are not adequately separated geographically. In this case, one would also need to carefully select the place of cultivation to avoid hybridization.

Df 4 Code. Wild relatives and/or their feral populations not frequent in the wild but well distributed over whole regions in Ukraine are in this category. The chances of hybridization with wild populations are significant, but under favorable conditions it can still be prevented. In this case, one also must carefully select the place of cultivation to avoid hybridization or any ecological effect. These require detailed biogeographically research.

Df 5 Code. These are wild relatives and/or their feral populations common in the wild and well distributed over whole regions in Ukraine. To prevent hybridization is almost impossible. In exceptional cases, it is still possible to choose a place for growth, to avoid hybridization, and environmental weed effects. To achieve this goal requires detailed biogeographical research.

With these three codes, you can make a preliminary rough risk assessment of the transgenic plants for wild relatives.

It was proposed by developers of this technical approach (Ammann et al., 1996; de Vries, 1996; de Vries et al., 1992) to establish six categories of probabilistic risk for use of transgenic plants.

First Category—No Effect

No related species or sexually compatible related species of the crop are known in Ukraine. Field releases of species belonging to this category are possible without any containment or short-term monitoring. Certain trangenes have to be tested in medium-term field experiments regarding their secondary effects on ecosystem.

This concerns such genes as genes for resistance to insects and pests.

Second Category—Minimal Effect

No records of spontaneous hybridization between the crop and the wild relatives are known in Ukraine. Field releases are possible after thorough clarification of the biogeographical situation. Short-term monitoring in confinements should be done prior to large-scale field releases.

Certain trangenes have to be tested in medium-term field experiments regarding their secondary effects on ecosystem (pest- and insect-resistance genes).

Third Category—Low but Local Effect

Gene flow occurs toward wild or feral species existing outside agricultural environment and control. Release experiments first should be done in confinement and then closely monitored in small-scale release.

This statement is restricted to transgenes not causing enhanced competitiveness in an outside agrosystem, such as herbicide tolerance. Any other transgenes should be carefully tested in confinements.

Fourth Category—Substantial but Local Effect

Gene flow is high and substantial, but still local and controllable.

Field releases could be done within strict confinements. A case-by-case analysis—including potential effects of the transgene—is required before any field releases are done. Long-term monitoring of field releases under strict biological or geographical confinement conditions is necessary in order to study competitiveness of transgenic crops. Risky transgenes have to be avoided.

Fifth Category—Substantial and Widespread Effect

Gene flow is high, substantial, and widespread and will not be controllable by any means.

No field releases of species belonging to this fifth category are possible. Medium-term monitoring under strict confinement condition is necessary in order to find out about competitiveness of transgenic varieties. Experiments with less risky crop varieties (e.g., with male sterility) having the same favorable effect desired.

Sixth Category—Unknown (One of the Three Indices is Unknown)

More studies are needed before any field releases are done.

Based on the proposed system, we calculated the risk category for some most important (oilseed rape, sugar beet, potato, and corn) and perspective crops (false flax, *Camelina sativa*) for Ukraine. Some of them—oilseed rape, sugar beet, potato, and corn—were under field trials in Ukraine earlier (Blume, 2000). Their dispersal index and risk categories are given in Table 1.

According to these data, potato and corn are classified as having "no effect." This means that in Ukraine there are no sexually compatible crops. The release of such crops is possible without restrictions. One only needs to pay attention to the possible secondary effects on the ecosystem.

Sugar beet is classified as having "minimal effect." This means that in Ukraine no records of spontaneous hybridization between this crop and the wild relatives exist. Field releases are possible after thorough clarification of the biogeographical situation. Before the large-scale release, short-term monitoring in confinement should be done, and certain trangenes have to be tested in medium-term field experiments regarding their secondary effects on the ecosystem (pest- and insect-resistance genes). Experiments carried out in climatic conditions of Ukraine showed that sugar beet can hybridize with their wild relatives *Beta maritima* only through artificial synchronization of flowering time. Thus, received hybrids behaved like a two-year plant that is showing no signs of weeds. In addition, they do not survive the winter in Ukraine (Negretsky, Novozhylov, & Blume, 2008).

Table 1. Dispersal index and risk categories of most important crops in Ukraine.

Species	English name	Dispersal index Dp, Dd, Df	Risk category
Brassica napus	Oilseed rape	4, 4, 4	Substantial but local effect
Beta vulgaris	Sugar beet	2, 2, 2	Minimal effect
Solanum tuberosum	Potato	1, 0, 1	No effect
Zea mays	Corn	0, 0, 1	No effect
Camelina sativa (L.) crantz	False flax or camelina	3, 4, 4	Substantial but local effect

Oilseed rape is classified as having a "substantial but local effect." Before any field release, each individual case needs to be analyzed, including the possible effects of the transgene. Long-term monitoring of field releases under strict biological or geographical confinement condition is necessary in order to study competitiveness of transgenic crops. Risky transgenes have to be avoided. In our case, transgenic oilseed rape were resistant to the herbicide therefore do not raise competitiveness outside agricultural systems.

Camelina is classified as having a "substantial but local effect" also. It will be necessary to carry out medium-term monitoring under strict confinement conditions in order to find out about competitiveness of transgenic varieties and experiments with less risky crop varieties (e.g., with male sterility).

Until now, in Ukraine there was no testing of higher risk category transgenic species. However, there is a test procedure for these plants, which was proposed by Fredshavn and Poulsen (1996). According to this method, studies of competitiveness in confinement (i.e., in a greenhouse) are necessary. This determines the competitiveness and the relationship between the normal flora and transgenic plants. But after this preliminary research, it will be impossible to identify all the possible consequences of the field release of transgenic plants, but one can check the transgenic plants during critical periods of development and compare them with existing non-transgenic plants. Thus, it is possible to determine all fundamental changes in biological behavior of transgenic plants.

If these experiments do not reveal any significant changes in the competitiveness of transgenic crops, field experiments on the methods proposed Rissler and Mellon (1993) can begin. This analysis technique involves a three-step evaluation of the possible formation of weeds as well as gene transfer.

The first step is determining the possibility of crop plants becoming weeds. This step allows the crops to be split into two categories of risk—lower and higher. The higher category includes plants that grow like weeds or have close relatives. These plants are subjected to the standard scheme of the experiments, while the representatives of the lower risk category are subjected to reduced procedures.

When carrying out risk-assessment experiments of transgenic plants in the higher category it is necessary to obtain answers to these questions: Do transgenic plants replace populations of non-transgenic plants? If not, the analysis stops. If yes, experiments must be carried out to study all environmental conditions in populations where transgenic plants replace non-transgenic plants. These results determine whether increased weediness exists in transgenic plants. If not, the analysis stops. If yes, long-term, small-scale experiments are carried out in strict confinement conditions to study weediness in different environmental conditions. If thus obtained disappointing results is prohibited commercialization of such forms.

The second stage is an experimental assessment of the possibility of the transgene's flow and viability of fertile hybrids between transgenic crops and their wild or weedy relatives. If hybrids are not formed, transgenic crops are low risk in terms of gene transfer and do not require further research. If the hybrid is formed, the analysis passes into the next stage. At this stage, the researcher must also obtain the answers to some questions, and if the result is positive, the analysis must continue.

1. Is it possible to outbreed with relatives in Ukraine?
2. Does the selective system support the gene flow, and in what direction?
3. Does the flowering period overlap or is it nearby?
4. Do transgenic plants and their wild relatives use the same methods of pollen dissemination?
5. Is there a natural cross-pollination between transgenic plants and their wild relatives if fertilization occurs and the formation of fertile seeds?

The second stage involves a rather simple experiment, which is conducted to assess transgenic crops compared to non-transgenic.

The third stage of the analysis must demonstrate that transgenic crops are better in terms of environmental tests than those with no transgene, as well as that they did not pose a risk as a weed in commercial use. If this has not been possible to demonstrate, the use of such transgenic crops is impossible. This step completes the risk assessment of vertical gene flow.

Proposing a Ukrainian version of preliminary risk assessment of the transgenic plants, we would like to demonstrate that this system first proposed by F.T. de Vries (de Vries, 1996; de Vries et al., 1992) and developed then by K. Ammann for Switzerland (Ammann et al., 1996) would be appropriate to use for the introduction of new crop plants based on regional principle. Naturally, it is not possible to arbitrate the crops and their wild relatives on one and the same level all over Europe. Biological behavior of the same species in different geographic conditions is sufficiently different. Risk assessment has to be done on a regional scale, taking into account local environmental conditions, species, and transgenes. There is no great hope to solve problems in risk assessment with one consensus technique, but further development of such techniques can gives us tools to common approaches for risk assessment of GM plants.

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