Marketing PGS' Genetically Modified Crop Plants including Rape Seed

P. L. J. Rüdelsheim[1]

Abstract

By early 1995, Plant Genetic Systems N.V. (PGS) obtained in Canada all approvals for cultivation of oilseed rape (*Brassica napus*) hybrids and consumption of derived products. The production of these hybrids is based on a new hybrid system, developed and introduced by PGS using genetic engineering. In order to obtain these approvals, specific research was conducted in support of the environmental impact assessment as well as the demonstration of substantial equivalence. The experimental steps for the characterization are summarized, including molecular aspects, agronomic evaluation, designs for ecological interaction and confirmation of nutritional wholesomeness. For some experimental approaches, e.g. on ecological interactions, experiments needed to be designed or tailored for assessment of oilseed rape. Based on this original work, routine tests have been developed. The scientific part of the presentation will be finalized with an overview of how the assessment of biosafety aspects is now fully integrated in these development schemes. The Canadian approvals were a first confirmation of the validity of the biosafety claims. PGS' hybrid oilseed rape was further cleared for growing in Europe and for import in the USA. All submissions have been made to possibly allow import in Japan. In other areas of interest, submissions have been prepared either for import or for cultivation. The main elements of the data package are applicable in all regulatory procedures. It is therefore anticipated that based on the first products, further international harmonization and eventually recognition of assessments will be realized.

Finally, information will be presented on the public interest in these developments. While different types of response - ranging from interested curiosity to political commitment - are recognized in every area of the world, the way public interest is shown is more related to cultural background. Appreciating these differences is a prerequisite for appropriate communication. We will give some examples of the PGS' communication scheme, which is aimed at listening to questions and concerns while responding in a very open and respectful atmosphere.

The first oilseed rape hybrids are now growing over a large acreage in Canada. PGS 3850 and PGS 3880, were recognized by the Western Canada Canola and Rapeseed Recommending Committee as top varieties. The Canadian farmers - and later also the European - will benefit from the improved agronomic performance. Through more uniform and reliable harvest of high quality oilseed rape, also importing countries will

[1] Director Regulaatory Affairs, Plant Genetic Systems, N.V., J.Plateaustraat 22,9000 Gent, Belgium.

enjoy the introduction of hybrid oilseed rape.

In addition to hybrid oilseed rape, PGS is active in developing the hybrid system and insect tolerance in corn and vegetables.

These projects are also at the verge of market introduction and the appropriate regulatory procedures have been initiated.

Introduction of a new hybrid system in oilseed rape

1 Developing the first oilseed rape hybrids based on the PGS SeedLink™hybridization system

A new type of male sterility and restoration has been developed by PGS using molecular biology techniques and has been introduced into oilseed rape. This new technology is generally designated by the trademark SeedLink™.

The male sterility of SeedLink™ is related to the expression of a natural catabolic enzyme (an RNase) in the tapetum cell layer of the anthers prior to pollen development. Since the enzyme inactivates the tapetum cells, it blocks the pollen production, resulting in male sterility of the flowers.

Restoration of fertility is obtained after crossing with a plant expressing (also in the tapetum) a highly specific inhibitor of the RNase.

Both the male sterility gene and the restoration of fertility gene are inherited as single dominant mendelian factors and are linked in the plant genome to a selectable marker gene conferring tolerance to a commercially available herbicide (active ingredient glufosinate-ammonium). This genetic linkage offers a very easy tool in breeding schemes and seed production. In fact, these dual gene constructs allow, in breeding programs, to select for plants carrying the male sterility or the restoration of fertility genes before the flower phenotype is revealed. SeedLink™ also creates a unique opportunity to devise production schemes which will guarantee the production of very pure hybrid seed lots.

Field experiments conducted for five years in more than 10 countries and over 100 locations demonstrated that SeedLink™ technology is very stable under different environments, does not exert any adverse biological effect and offers a full restoration in the genotypes which have been extensively tested so far.

2 Development of a research program on biosafety aspects

By early 1995, PGS obtained in Canada all approvals for cultivation of oilseed rape *(Brassica napus)* hybrids and consumption of derived products. In order to obtain these approvals, specific research had been conducted in support of the environmental impact assessment as well as the demonstration of substantial equivalence. For this overview, we classified some of the experiments in categories of molecular characterization,

agronomic performance, environmental impact and food/feed uses.

1) Characterization of the transformation events

At the molecular/biochemical level, the transformation events were characterized in detail. Table 1 gives an overview of the main results from a large series of analyses. In addition to characterization of the lines, which allows further tracking in development schemes and registration processes, the molecular specifications confirmed that the expression patterns were as predicted and that no other products were expressed inadvertently in the transformants.

Table 1 PGS Hybrid Oilseed rape - Molecular characterization

	MS1	RF1
Copynumber	1	1
Rearrangements	no	no
Only T-DNA transferred	yes	yes
Insertion site characterized	yes	yes
Only genes of interest expressed	yes	yes
Specific identification	yes	yes
Genetic stability	yes (> 5)	yes (> 5)

2) Agronomic performance

After *in vitro* selection, plants were transferred and upscaled in the greenhouse. Essential parameters such as plant morphology, linkage between the level of glufosinate tolerance and flower phenotype, a predictable segregation pattern and if applicable, the capacity to restore the fertility of a range of male sterile oilseed rape plants, were monitored to select candidate male sterile and fertility restorer plants. The transgenic oilseed rape lines were identical with the untransformed control plants for all biological features except for the phenotype due to the inserted genes.

From 1991 onward, the new hybrid system was evaluated under open field conditions demonstrating the feasibility of the system and conformity to the scientific expectations. Data were gathered on the performance of the male sterile system and on the methodology to upscale the amount of hybrid seed necessary for consecutive evaluation trials. The entire field program with the hybrid system in oilseed rape covered releases in Belgium, France, Canada and Sweden. The stability of the nuclear male sterility and fertility restoration was confirmed in different genetic backgrounds as well as under different environmental conditions.

In 1992, the program was intensified with the evaluation of the feasibility of the system in real agronomic and breeding perspectives at approximately 15 test locations

representing multi-site trials established in several countries throughout Europe and North America.

- Stability of the flower phenotype throughout the entire season under diverse environmental conditions,
- performance of the selectable marker in agronomic treatments,confirmation of the absence of yield penalties associated with the selected lines and stability of the gene expression in different backcrossed genetic backgrounds were studied.

In 1993, various multi-site trials were performed in several countries throughout Europe and North America. Results of the oilseed rape field trials confirmed that a reliable hybrid system in oilseed rape had been obtained. In 1993, a first spin-off breeding group started to incorporate the new hybrid system in a focused breeding program.

Henceforth, the lines have been incorporated in a testing program covering Europe, North and South America and Japan.

3) Environmental interactions

The major topic of investigation is whether genetically engineered organisms or the transgenes they harbor may become established in new environments where they could disrupt ecological systems. With reference to the new hybrid oilseed rape system, the question can be asked whether the genetically engineered oilseed rape may invade and disrupt agricultural or natural communities.

Though no negative impact of the transgenic oilseed rape plants on the environment has been detected in the extensive field trials, several possible environmental interactions between transgenic plants and the environment have been suggested by scientists, regulatory organization and/or environmental interest groups. Additional experiments were performed to address the potential environmental impact of the hybrid oilseed rape plants to answer the following questions :

- how likely is the inserted genetic material to provide a selective advantage to the transformed oilseed rape plant so that it might become a weed in agriculture or invade natural habitats ?
- will the transgenic oilseed rape plant have any negative impact on organisms of different trophic levels that come in direct contact with the transgenic plant ?
- are the transgenes likely to be transferred via pollen to other plant species and will these plants species then become more weedy or more invasive ?

In natural as well as in arable areas, there are no indications of altered weediness and/or invasiveness of the transgenic oilseed rape, harboring the new hybrid system versus the non-transgenic oilseed rape as :

- the mechanisms and likelihood for dispersal of the plants are similar to what is known today from varieties;
- the colonization and establishment capacity has not changed through the introduction of the genes involved in the hybrid system;
- oilseed rape is a crop plant, needing agricultural management in order to yield some

offspring; in "wild", unmanaged environments, oilseed rape is a poor competitor, susceptible to a wild range of pests; the newly introduced genes did not increase the ability of the plants to compete;

· in combination with the use of glufosinate-ammonium, several scenarios can be reviewed in terms of competitive advantage; based on the application of glufosinate-ammonium, the creation of a new niche for the genetically modified lines is unlikely;

· though non-transgenic as well as transgenic oilseed rape can be a volunteer in subsequent crops, current agricultural practices (rotation, selective herbicides, herbicide-resistant oilseed rape developed by classical breeding, isolation of production fields of different rapeseed types) are able to control non-transgenic and transgenic oilseed rape volunteers.

While the competitive behavior of the genetically modified plant was not changed, further observations focused on direct and indirect interactions of the plants with other environmental components. For instance, a number of experiments were conducted to investigate the possible impact on pollinating insects (bees) that forage on the plants, and on birds and mammals, known as nuisances in agriculture. In all the studies the response of the test systems was similar to that of transgenic and non-transgenic batches of oilseed rape.

With the widespread presence of *Brassica* species established, a great deal of attention was focused on the possibilities for outcrossing. All information from the literature, larger Risk Assessment programs (e.g. the EU-programs BAP and BRIDGE) and PGS research were integrated into an introgression model. This model takes into account the fact that the free movement of genes between related species is hindered by three levels of barriers. The first barrier, a physical barrier, indicates that in order to have a cross between two species there needs to be a physical exchange. Species that flower at distinct times or in distinct regions can be regarded as physically separated. A second barrier is of a genetic kind, implying that some genetic combinations can not survive, nor lead to mature seeds or fertile plants. In many risk assessment studies, advanced techniques have been used to circumvent such barriers, enabling at least an approach to a worst case scenario. Finally, since the intermediate hybrids have to compete with the wild type parental species and the rest of the vegetation, one needs to recognize an ecological barrier. Based on these three types of introgression barriers, it can be deduced that although introgression from the traits from *Brassica napus* to *Brassica campestris* and *Brassica juncea* is technically possible, complete introgression under natural conditions is very unlikely. Even in this unlikely event, it was demonstrated that the traits of the hybrid system would not affect the environmental behavior of those plants. Or to rephrase this statement, the introgression would not make the wild relatives more noxious, uncontrollable weeds.

3 Integration of screening and biosafety research in future development programs

The results described above were obtained essentially in projects with the first transformation events. PGS continues to produce and screen new transformation events, broadening the genetic basis of the inserts and improving the system. Based on the international review of the data-package, a standard set of experiments has been incorporated in the development and screening program for the new lines. Early stages involve molecular characterization, performance evaluation and plant morphology, later complemented with food and feed wholesomeness determination, competitive behavior analysis and confirmation of stability over a wide range of environments and genotypes. By the time an event is recognized as an "elite allelle" and can be introduced with confidence in a breeding program, the regulatory package is ready for review. As more experience is gained through the different regulatory review systems, the set of experiments and checks is adapted. With this integration in the selection scheme, PGS aims at developing new materials which are performing according to the highest quality levels, while ensuring that they are acceptable within the most stringent regulatory requirements.

Experiences with regulatory review systems

The Canadian approvals were a first confirmation of the validity of the biosafety claims. PGS' hybrid oilseed rape was further cleared for growing in Europe and for import in the USA. All submissions have been made to allow import in Japan. In other areas of interest, submissions have been prepared either for import or for cultivation. The main elements of the data package are applicable in all regulatory procedures. It is therefore anticipated that based on the first products, further international harmonization and eventually recognition of assessments will be realized.

Experience with public interest

While different types of responses -ranging from interested curiosity to political commitment- are recognized in every area of the world, the way public interest is shown is clearly related to cultural background. Appreciating these differences is a prerequisite for appropriate communication. PGS' communication approach is based on listening to questions and concerns while responding in a very open and respectful atmosphere.

At this stage of first commercial introductions, PGS communication has shifted to demonstration of specific applications of biotechnology. Since 1995, a large number of demonstration sites have been set up in western Canada allowing farmers, consumers and special interest groups to get an immediate look at the plant material, the performance and the particular aspects. In 1996, the first field trials in the framework of an EU-funded project FACCT aimed at bringing biotechnology closer to the public,

were performed in Europe. Field days enabled representatives of different areas, including farmers, extension workers, processing industry, consumer organizations, authorities and press to have an open dialogue on the achievements and challenges. This communication effort aims at making ag-biotech a concrete, tangible principle. Based on such principles further exchange of information and dialogue will be more focused and on an equal level between all participants.

Approaches for other PGS products

The first oilseed rape hybrids are now growing on a large acreage in Canada. PGS3850 and PGS3880 were recognized by the WCC/RRC as top varieties. The Canadian farmers -and later also the European- will benefit from this development, but through more uniform and reliable harvest of high quality oilseed rape, also importing countries will enjoy the introduction of hybrid oilseed rape.

In addition to hybrid oilseed rape, PGS is actively developing the hybrid system and insect tolerance in corn and vegetables. Some of these projects are also at the verge of market introduction and the appropriate regulatory procedures have been initiated.