Information for the Public Register

Syngenta Mogen B.V

Comparison of potatoes, genetically modified to express an enhanced resistance to fungal pathogens with existing non-modified varieties, grown under standard agronomic conditions

European Notification number
B/BE/01/V2

Taking into account the advise of the Biosafety Council and the Service of Biosafety and Biotechnology of the Scientific Institute of Public Health – Louis Pasteur, the Belgian Ministry of Agriculture has given permission to Syngenta Mogen B.V. to carry out field trials in the year 2001, as described in application B/BE/01/V2.

This programme will be carried out on one experimental site in Flanders and will be carried out on territory of the municipality of Ravels. This programme will follow the normal culture period of the potato (*Solanum tuberosum*) that runs from the month of June till November 2001.

<u>Responsible person to be contacted for further information concerning the trials:</u>

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1. Description of the genetically modified plants.

<u>Novel trait(s) of the genetically modified potato plants:</u>
The intended effect of the introduced genes is to increase the resistance of the potato plants against fungal diseases. In particular the resistance against the late blight pathogen *Phytophthora infestans* will be increased.
In addition, a selectable marker gene is present, which allows the selection of modified cells in tissue culture after transformation. Plantsexpressing the selectable marker gene are resistant to the antibiotic kanamycin.

The Latin name of potato is *Solanum tuberosum* sub-species *tuberosum*. The varieties used for the transformation are ‘Bintje’, which is widely grown in Europe and ‘Russet Burbank’, a major variety in the United States of America. Both varieties are susceptible to late blight.

<u>What is genetic modification of plants:</u>

In all living organisms, micro-organisms, plants, animals and humans heritable traits are encoded by their genome. Each cell of an organism carries a complete copy of this genome. In plants, as in humans and animals, the genome consists of a number of chromosomes, which are located in the nucleus of each cell. Chromosomes are in fact long chains of DNA, which are folded and packed in proteins and reside in the cellular nucleus. Discrete parts of these DNA-chains form the so-called ‘genes’ (single: gene). When a gene is active it produces a protein, which has a specific function. Each gene carries, so to speak, the recipe for the production of a protein. For example, the function of a protein might determine a morphological characteristic of a plant or its function might be to control an essential process in the cell, like photosynthesis. Plants carry more than 20 thousand genes. In many cases,, but by far not all, it is known which protein recipe the gene carries and what the function of that protein is.

In plant biotechnology under laboratory conditions, an extra piece of DNA, carrying a gene or several genes, is inserted in the nucleus of a plant cell by making use of a so-called ‘transformation process’. Under laboratory conditions a complete plant is regenerated from such a transformed plant cell. As a result each cell of this plants contains the extra piece of inserted DNA. A gene located on that extra piece of DNA provides the transformed plant with the recipe for a protein that was not produced in the original plant. In this way the plant obtains a novel trait and is called ‘transgenic’ or ‘genetically modified’.

<u>Description of the inserted genes and their mode of action.</u>

The modified potato plants, which will be tested in this field trial, contain an extra piece of DNA carrying three genes.

Selectable marker gene: This gene renders the recipient plant cells resistant to the compound ‘kanamycin’. The selectable marker gene allows the selection under laboratory conditions of plants cells that have taken up the extra piece of DNA after the transformation process. This was accomplished by growing plant tissue on medium containing kanamycin. Plant cells that have not taken up the extra piece of DNA will not survive on the growing medium when exposed to kanamycin.

Resistance gene: The second gene is a resistance gene from tomato. This gene is called ‘*Cf-9*’ and convers resistance to the fungus *Cladosporium fulvum*, a tomato pathogen. In nature *Cladosporium fulvum* excretes a small protein (or peptide) called ‘AVR9’, when it infects tomato leaves. The *Cf-9* gene carries the recipe for the production of the CF9-protein. In tomato varieties carrying the *Cf-9* gene, cells in the leaves of the plant contain the CF9-protein. The CF9-protein can sense the presence of the AVR9-peptide and thereby the presence of the pathogenic fungus. When a CF9-protein senses the presence of an AVR9-peptide, a signal is given to the plant cell and a defense response is induced. This defense response is called the ‘hypersensitive response’. This hypersensitive response limits further growth of the invading fungus.
The modified potato plants carrying the extra piece of DNA also contain the *Cf-9* gene. The cells of the modified potato plants now have the ability to produce the CF9-protein, just like

the tomato plants that inherently carry the *Cf-9* gene. The cells of the modified potato plants are therefore also able to sense the presence of the AVR9–peptide and to trigger the hypersensitive response as soon as the AVR9-peptide is present.

Avirulence-gene: The third gene present on the inserted piece of extra DNA is the *Avr9* gene, which originates from the fungus *Cladosporium fulvum.* This fungus is pathogenic to tomato, but not to potato. The *Avr9* gene carries the recipe for the production of the AVR9-peptide (a peptide is a small protein). The fungus normally produces this peptide as soon as it infects tomato leaves. The modified potato plants, that carry the extra piece of DNA, now also posses the *Avr9* gene and are therefore able to produce this fungal AVR9 peptide themselves. As soon as the CF9 protein and the AVR9 peptide are produced simultaneously in the potato leaf tissue a signal is sent to the cell and the hypersensitive response is induced.

Promoters: A gene is only active when it is switched on. Whether a gene is active or not is controlled by a part of the gene that is called the ‘promoter’. The promoter of a gene serves as a switch. Under which circumstances a promoter switches the gene ‘on’ or ‘off’ is depends on the nature of the promoter. Some promoters are always ‘on’ in all cells and are called ‘constitutive’ promoters. Other promoters are only ‘on’ in specific tissues like roots or leaves. Certain promoters are switched on by specific stress conditions, like drought. Also promoters exist which are typically switched on after infection by a pathogen, like a fungus. These last type of promoters are called ‘pathogen-inducible promoters’. In the modified potato plants either the *Cf-9* or the *Avr9* gene are under the control of a pathogen-inducible promoter. The other gene is controlled by a constitutive promoter and therefore always active.
As soon as a fungus, for instance *Phytophthora infestans*, infects a modified potato plant, the pathogen-inducible promoter will switch on the gene it controls and the subsequent protein (or peptide) will be produced. In this way both the *Cf-9* and the *Avr9* gene are only active simultaneously after infection. Subsequently both the CF9-protein and the AVR9-peptide are only present simultaneously after infection. The CF9-protein will sense the simultaneous presence of the AVR9-peptide and subsequently a signal will be send to the plant cell which will trigger the hypersensitive response. This hypersensitive response will limit further infection by the invading fungus.

2. Objective of the field trial:

The objective of the trials is a primary evaluation of resistance against fungal diseases of a selection of modified potato lines under field conditions. In particular, resistance against the late blight fungus *Phytophthora infestans* will be assessed. This will done by comparing the progress of the disease in the selected genetically modified lines with that in the original non-modified varieties and a set of standard varieties with different known resistance levels. Further more crop development and tuber production will be assessed and compared to that of the original varieties.

Field trials are required to assess the value of the increased disease resistance for the farmer, since crop development can differ under field conditions compared to the controlled

conditions in the greenhouse or in growth rooms. In addition, the fungus will behave differently in the field compared to controlled conditions.
'Field resistance' and agronomic features, like crop development and yield, can therefore only be evaluated properly under field conditions. Field trials are therefore a prerequisite to assess the true value of the modification for the agriculture practice.

In addition field trials are required to monitor for possible unintended effects, which might occur in the field. The trial is for research purposes only.

3. Advantage for the environment, the farmer and the consumer.

Late blight is one of the most important fungal diseases in potato culture. The disease was responsible for the complete loss of the potato yield in Ireland in 1845. As a result a great famine occurred during which many people lost their lives. Since that time the disease has persisted in Europe, but during the 20th century, through the development of effective fungicides, farmers were able to improve on the control of the disease,.. Since the 1980's however more aggressive strains of the fungus have spread throughout Europe. By the application of sanitary measures, implementation of late blight alert systems and targeted application of modern fungicides the disease is currently often controlled at an acceptable level. However during seasons with weather conditions favourable for disease development (cool and wet) great losses due to late blight do occur. Development of potato varieties with increased resistance to the disease will make the application of the measures mentioned above more effective and more durable. For the farmer the chances of large economical losses due to the disease will decrease substantially. Also in the longer term the disease will be more controllable. When culturing potatoes with an increased resistance level, crop protectants can be applied in a very efficient way, which will lead to a reduction in use and decrease the dependability on agrochemicals. Classical breeding of potato is a time consuming process. The development of new potato varieties with both increased resistance to late blight and the desired characteristics for processors and consumers therefore progresses at low speed. Molecular breeding through genetic modification can contribute considerably to the development of varieties with the desired combination of traits.

4. Biology and live-cycle of the plants used.

4.1. Weediness of the plants used:

Wild potato species occur in the mountains of Central and South America. In Belgium the potato only occurs in managed agricultural environment and its survival is fully dependent on human activities. It is unable to persist in natural habitats. Potato plants are sensitive to drought and to frost and therefore will mostly not survive the winter season. Tubers remaining in the ground after harvest might survive a mild winter leading to so-called volunteers in the next season. Potato volunteers are however routinely controlled by conventional agricultural practices. Potato plants do not occur as weeds under Belgian conditions. It is not expected that the modified potato plants will behave differently in this respect.

4.2 Survival and dissemination of seeds and tubers.

Flowers are the sexual reproductive organs of a plant. Pollen forms the male reproductive cells. After pollen has landed on the female parts (the pistil) it can germinate, penetrate and fertilise the female reproductive cells. Fertilised cells will from a seed inside what is called "the fruit". Seeds can easily be observed when a tomato fruit is cut open. When a potato plant is flowering, self-pollination (i.e. the plant fertilises itself) can occur. This actually occurs in 80-100% of the cases. Also some cross-pollination can occur (i.e. pollen from one plant fertilises a flower of another plant). After fertilization, seeds develop in a small fruit that looks like a small green tomato fruit. Potato seeds can survive in the soil. Under favourable conditions they will geminate and produce a plant. However, the farmer will treat these plants in the same way as a volunteer plant grown from a tuber and hence they will be eliminated by normal agricultural practice. Plants from true potato seeds might only be observed in managed agricultural land. In non-managed ecosystems they will not be able to compete with the natural vegetation.
The potato varieties that have been modified are 'Bintje' and 'Russet Burbank'. Both flower poorly and almost never form viable seed. It is not expected that the modified plants will differ in their inability to form viable true seeds.

Tubers which might be left behind and remain in the ground after harvest normally will not survive the succeeding winter due to frost. Tubers that might survive a mild winter will sprout the next season and form potato plants. These plants are called potato 'volunteers'. Potato volunteers are effectively controlled in conventional agricultural practice. It is not expected that the modified potato plants will differ with respect to their vulnerability to frost and drought or that they will be less susceptible to conventional volunteer control measures. This will be verified in the season following the field trial. The site of introduction will be monitored for volunteers. Each volunteer that will be found will be destroyed.

5. Possible effects on or risks to the environment:

5.1. Cross-pollination and invasion of natural ecosystems:

Dissemination of transgenic pollen resulting in cross-pollination:
The chance of dispersal of the transgenes outside the site of introduction via cross-pollination is practicably speaking zero. Cross-pollination of potato plants can occur via insects, but is restricted to very short distances. In field studies transfer of transgenes via cross-pollination was found at very low frequencies at a distance of 10 meters, while at a distance of 20 meters no evidence of cross-pollination was found. The distance from the field trial to the nearest potato field will be at least 40 meters. Cross-pollination with the potato related weed species *Solanum nigrum* (black night shade) and *Solanum dulcamara* (bittersweet) has not been shown to occur.

Dissemination of transgenic seeds and/or tubers:
The potato varieties used for the modification, 'Bintje' and 'Russet Burbank', only flower occasionally and rarely produce true potato seed in the field. If true seed, is produced, it is able to survive in the soil and has the potential to produce volunteers. However, the volunteer plants from both the modified and non-modified seeds will be unable to establish themselves

in the environment. Potato plants from true seed can occur only on cultivated land. In natural habitats these plants do not survive since they are unable to compete with the natural vegetation. Potato plants from true seed occurring on cultivated land will be recognised as potato volunteers and treated accordingly. Potato volunteers are routinely and effectively controlled under normal agronomic practices.

Selective advantage:
Resistance against kanamycin will not give a selective advantage to the modified plants, since potato plants are not treated with this compound under normal agricultural conditions. The competitive advantage of the increased resistance to fungal diseases will be very limited. Survival on farmers land is restricted mainly by the sensitivity of the plants to frost and drought and not by their susceptibility to disease, It is not expected that the modified plants will be less sensitive to frost or drought than non-modified potato plants. Furthermore, potato volunteers are systematically controlled using methods to which the modified plants are expected to be equally sensitive as non-modified plants. Their inability to invade natural habitats will also be determined by the sensitivity of the potato plants to frost and drought and this will not change as a result of increased disease resistance.

5.2. Interaction with target organisms:

The intended effect of the modification is increased resistance to pathogens including the fungus *Phytophthora infestans*. In fact *Phytophthora infestans*, the causal agent of potato late blight, is the main target organism. When not controlled, this disease can completely destroy a potato crop. In Europe the importance of the late blight disease has increased considerably over the last 20 years. Reduction of its causal agent is therefore desirable.

5.3. Interactions with non-target organisms:

Field trials of similar modified potato plants have been carried out previously in the United States, the United Kingdom and the Netherlands. No adverse effects on non-target organisms were observed. Also during the planned field trial in Belgium close observations of the modified plants will be carried out to check for the occurrence of unexpected interactions with non-target organisms that can be detected by visual observations.
There is strong evidence that in *Cf-9* carrying plants the hypersensitive response induced by the AVR9 peptide does not occur in the roots. Therefore it is highly unlikely that the hypersensitive response will affect either soil-borne pathogens or other (beneficial) soil micro-organisms, like endomycorrhizae. Detailed studies of the interaction between modified potato plants with enhanced disease resistance and the soil micro-organisms can be conducted in a later stage, as soon as lines showing a robust increase of disease resistance under field conditions are selected. In this respect one should realise that also the culture of a non-modified field crop has a tremendous impact on all other living organisms on the same field. For example, a wheat crop will attract different insects to a potato crop. Also the composition of the soil micro-flora and micro-fauna is strongly affected by the cultivated crop. Also agricultural measures such as ploughing or application of manure will impact on the organisms living in the soil.

5.4. Impact of large-scale and long-term use:

Data on the impact on the environment and public health of large-scale and long-term use of the modified plants are not available at this stage. Since the intended release is of a small scale and for research purpose only these data are not relevant.

6. Containment, control and monitoring measures:

6.1. Monitoring of pollen dispersal:

As described above it is highly unlikely that gene transfer through pollen dispersal will occur as a result of this trial. The varieties used only flower occasionally and will produce only little viable pollen. Furthermore an isolation distance of 40 meters will be applied. Cross-pollination of wild sibling species has not been shown to occur.

6.2. Monitoring of dispersal of seeds and tubers:

Also the chance of dispersal via true potato seed is effectively zero. Both varieties used rarely produce viable seeds. Any seed that might remain on the site of introduction that might survive will produce a volunteers plant in the next season and will be controlled effectively similar to volunteer plants emerging from tubers that might be left behind after harvest and that do survive the succeeding winter.

6.3. Post harvest treatment:

The tubers will be harvested, packed in closed bags or containers and will be destroyed. Machinery used for harvesting will be inspected visually and cleaned from any tubers before leaving the site of introduction. The desiccated haulms will be left behind at the site of introduction and will later be incorporated into the soil, according to common practice.

Tubers that may be left behind after harvest will be killed when frost occurs during the next winter. Tubers that may survive in case of a mild winter will produce volunteer plants. For one year after the trial has been harvested, the introduction site will be monitored and volunteer plants will be destroyed.

7. Monitoring:

In the succeeding season a crop distinct from potato will be grown at the site of introduction. This will allow effective control of potato volunteers. This will be achieved by visiting the introduction site several times in the next season. Volunteer plants will be monitored visually. Any occurring volunteer plants will be destroyed by treatment with a systemic herbicide or by manual removal, after which the plants will be packed in closed bags or containers and moved to an installation where they can be destructed by heating or incineration.

8. Destruction of the transgenic material:

The desiccated haulms will be left behind on the field and will be incorporated in the soil, according to common agricultural practice. Tubers will be harvested mechanically and collected in closed bags or containers. The harvesting machinery will be inspected visually and cleaned from any tubers before leaving the site of introduction. Subsequently the harvested tubers will be moved to an installation where they will be destroyed by incineration. None of the tubers will enter the animal feed or the human food chain.

9. Emergency plans:

If necessary the potato plants can easily be destroyed using a herbicide. During the trial individual plants can be removed in closed bags or container and destroyed by heat or incineration. When, in case of unforeseen circumstances, it would be necessary to terminate the entire trial this can be achieved by treatment with an appropriate herbicide. After premature termination of the trial removal and destruction of tubers will be carried in the same way as normally at the end of the trial. Since the trial will be visited regularly for monitoring and maintenance, it will be possible to react appropriately to unforeseen circumstance.

10. Inspection:

In Belgium the “Inspectie-generaal der Grondstoffen en Verwerkte producten van het Ministerie van Middenstand en Landbouw” is in charge of inspection of field trial with transgenic plants. In order to enable planning of the inspections the applicant is obliged to inform the inspection service on sowing/planting- and harvesting dates. In the field inspectors will check that the prescribed procedures will be followed during sowing/planting and at harvesting. Furthermore inspectors will take samples to be analyses in authorised laboratories.

11. Social-economic aspects:

The intended effect of the genetic modification is to increase the resistance of the potato plants to the late blight disease, which is caused by the fungus *Phytophthora infestans.*
Conventional methods to control this disease use the (often weekly) application of fungicides. Alternative technologies do not exist. Organic culture of potato in Belgian is problematic, due to high disease pressure in most years. By using existing varieties, that are less susceptible to the disease, the development of the disease can be slowed down to some extent.
Such varieties, however, produce tubers, which do not meet the standards of the processing industry and the consumer.
In many European countries, including Belgium, policies are developed to reduce the input of agrochemicals. This puts a lot of pressure on the use of these substances in potato culture. For a more sustainable potato culture, that is less dependent on the use of agrochemicals, it is

essential to develop alternatives. We believe the development of genetic modified plants will contribute strongly to this.

Syngenta takes a leading role in informing the public on the backgrounds, possibilities and advantages of plant biotechnology. In the United Kingdom Zeneca Agrochemicals (one of parent Companies of Syngenta) has successfully launched a genetically modified tomato puree, which exhibited better processing characteristics. The success of this market introduction was due to the efforts we took in communicating with the general public during the development of this product.

We are convinced of the advantages genetically modified potatoes with enhanced disease resistance will bring to the farmer, the consumer and the environment, as described above. The intended field trial with modified potato plants is an important link in the development chain that will lead to a marketable product.

We believe that via biotechnology we can develop product of high value to people both in the developed and the developing world. We feel responsible for the safety and the quality of our products. At the same time, we recognise the current public concerns associated with applications of biotechnology in food. Syngenta will continue to participate in the public debate on this issue and will take its social responsibility.

More information on Syngenta's vision on the potential of biotechnology can be found at http://www.syngenta.com/en/customer/biotech.asp.

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