PRAKTIJKONDERZOEK
PLANT & OMGEVING

Information for the public

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Limited field trial on the expression of virus resistance in the field in genetically modified, supposedly virus-resistant lilies.

European Notification number
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After advise of Biosafety Council and the Service of Biosafety and Biotechnology, Scientific Institute of Public Health - Louis Pasteur, the Belgian Ministry of Agriculture has allowed the Laboratorium voor Bloembollenonderzoek to carry out experiments in 2001 as described in dossier B/BE/01/V1.

This project will be carried out at one location of the Flanders and will be made in the territory of the local authority Melle. It also will follow the normal period of cultivation of lily (*Lilium longiflorum*) that runs from the month May till October 2001.

<u>Responsible person for contact about additional information concerning the experiments</u>:

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0. Contents:

1. Description of the genetically modified plants

Description of the starting material
Family: *Liliaceae*
Genus: *Lilium*
Species: *longiflorum*
Cultivar: 'Snow Queen'

Common name: lily, Easter lily

Description of the genetic modification
Using biotechnological methodologies (genetic modification) lily plants have been produced that were proven to be resistant to Lily Symptomless Virus (LSV) in glasshouse experiments. In these

transgenic lilies, a small piece of the virus DNA had been incorporated by which the plants became resistant towards the virus. The incorporated virus-DNA enables recognition of the virus as a being foreign. Then the virus is degraded by the plant.

During the process of genetic modification, it is essential to be able to distinguish transformed from nontransformed tissues. Therefore, together with the virus-DNA, in the lily genome genes have been incorporated that enable selection. Expression of these genes in the plant tissues renders the tissues resistant towards two antibiotics. So after the foreign DNA (virus DNA and the two genes that bring about resistance towards antibiotics) has been incorporated in the host-plant DNA, the tissue is cultured on medium to which this antibiotic has been added. Then, transformed tissue survives as it is resistant towards the antibiotic. This tissue also has the virus-DNA which is expected to bring about resistance to the virus.

It might be that the resistances towards antibiotics are transferred to malign bacteria via the soil or via digestion of the plant material (horizontal transfer) in the gastrointestinal tract of humans or animals. This may result in an increase of resistance of bacteria and thereby in a decreased usability of these antibiotics in human and veterinary medicine. However, such horizontal transfer from plants to bacteria has never been shown in nature, nor in culture under methodologically correct conditions. The chances that this may occur are generally thought to be negligible. Theoretically, though, such transfer may occur when the transgenic lilies are being cultured by farmers in very high numbers. In this experiment, only few plants are planted in the field. In addition, effective precautions have been taken (see below).

2. Aim of the experiment:

The sole purpose of the experiment is to examine whether the previously observed virus-resistance in the glasshouse is expressed under field conditions. Glasshouse experiments have been carried out before, but they can never completely mimic the conditions in the field. Because of this, field trials are necessary. Precautions will be taken that the lilies that are used in the experiment will not be sold, mixed with normal, commercial lilies or introduced in the environment in any other way. When the experiment shows that the virus-resistance is expressed to a sufficient extent in the field, these lilies will nevertheless not be used for introduction of virus-resistance in lily. In stead, new experiments will be done in which lily is transformed again, but in such a way that only the resistance gene(s) are incorporated and no selection genes.

3. Advantages for the environment, the farmer and the consumer:

In the cultivation of lilies, virus infection may cause a considerable reduction of yield and quality. Lily Symptomless Virus (LSV) does cause losses in yield up to 20 % and the quality of the flowers is much reduced. LSV is particularly problematic because there is no natural resistance against this virus in any lily genotype. Therefore, resistance cannot be introduced by crossing commercial genotypes with wild genotypes collected in their natural habitat. LSV is spread by aphids. Farmers now control the spread by aphids by spraying mineral oils or synthetic pyrethroïds. To enable sustainable cultivation of lily, the use of chemicals to control diseases should be reduced. Virus resistant lilies are of utmost importance because they do not require spraying of chemicals to control aphids. Reduction of the use of chemical control results in a more healthy environment.

4. Biology and life cycle of lily:

4.1. Lily as a wild plant:

L. longiflorum is an indigenous plant in Japan. It grows in a maritime, tropical climate. Lily is grown as an ornamental plant all over the world. Lily is a bulbous plant and may propagate both vegetatively via daughter bulbs or generatively via seeds.
In the field, lily produces 1-2 daughter bulbs per bulb per year. In addition, small bulblets may be formed along the stem, under the soil and occasionally in leaf axils. Bulblets may also be formed from detached scales. Both the dispersal of the large daughter bulbs and the small bulblets occurs very slowly. In contrast with, e.g., potatoes, no stolons are being formed.
L. longiflorum 'Snow Queen' is selfincompatible. This means that self-pollination cannot occur. Only at a continuous elevated temperature (± 26°C) self-pollination and subsequent seed formation may occur. In North-West Europe, though, these conditions only occur in the greenhouse and never in the field. Pollen is dispersed by insects. Likely, wild relatives of lily occur sporadically in Belgium. In The Netherlands and in Germany, two related lilies are found, *L. bulbiferum* and *L. martagon* (rare). These lilies likely also occur in Belgium but very infrequently. In addition, they are incongruent with *L. longiflorum.* The transgenic plants do not differ from the nontransgenic plants with respect to their reproductive behaviour.
In conclusion, spreading of the introduced foreign genes via bulbs or seeds is very slow or impossible, respectively. Apart from that, for determining virus-resistance it is not necessary that the plants flower. Therefore, in the experiment all flower buds will be removed long before they have opened.

4.2. Survival and disperse of the seeds:

Even though it is very unlikely that seeds are being formed, if this would happen seeds would germinate under the field conditions. However, seed dispersal is passive and the chances for survival of the seedlings are very small when no special measures are taken to stimulate growth (temparature and humidity) and to control of pathogens. Transgenic plants do not differ from the nontransgenic plants in this respect.

5. Possible risks for the environment:

5.1. Crossing with and introduction in natural ecosystems:

- Dispersal of transgenic pollen:

To determine whether the plants have acquired virus-resistance, they do not have to flower. To prevent flowering all flowerbuds are picked before they have opened and are then destructed. In the experiment, circa 1200 bulblets are planted at 20 m^2. Most of them will not flower because of their small size. Picking the flowers in time can easily been done. Thus, transgenic pollen will not be produced during this trial.

- Dispersal of transgenic seeds:

Because all flowerbuds will be destructed, no seeds will be formed.

- Selective advantage:

Dispersal of large daughter bulbs of lily occurs at a very slow rate, because they are formed underground in the mother bulb. The dispersal of these bulbs is prevented simply by cultivating them in containers. Small bulblets that have been formed on the stem and have possibly become detached, will be collected from the soil. In the years after the experiments, bulblets that have been overlooked and have not been killed by the spraying with Round-up (see below) will sprout and are then collected and destructed. Dormant bulblets cannot survive in soil for a long period without sprouting. This is in contrast with seeds that often remain in the soil as a seed bank. The reason for this is that in contrast with seeds, bulblets cannot be dried.

5.2. Interaction with target organisms:

Aphids fly from plant to plant and feed by sucking phloem sap. Hereby, they spread the virus. The aphids do not spread the genes that have been incorporated in the plant genome.

5.3. Interaction with nontarget organisms:

The transgenic plants do not constitute a risk for public health. The incorporated genes do not code for toxic compounds. So, if an animal or a human happens to consume plantmaterial, it will not suffer from harmful consequences. In Belgium, there are no native or foreign occurring plants that can be fertilized or ousted by lily.

5.4. Impact of use at a large scale and for a protracted period of time:

As indicated above, the sole purpose of this experiment is to check whether the virus-resistance that has been observed in the glasshouse, is also expressed in the field, so that lilies can be cultured in the future without the abundant use of chemicals. If the outcome is positive, lilies will be transformed again without using selection markers.

6. Measures taken for restriction, control and succession.

6.1. Control of the dispersal of pollen:

All flower buds will be picked and destructed before opening. The flower buds of lily are large and can be easily distinguished and removed. Thus, no spread of pollen will occur. In addition, there are no lilies cultured for miles around.

6.2. Controle of the dispersal of transgenic seeds:

See 6.1.

6.3. Conditions after harvest:

The bulbs are planted in plastic containers from where underground dispersal is impossible.
During the growing season, leaf samples are collected twice to examine the presence of virus. The bulbs and their roots will be collected from the soil at the end of the growing season (October) and assayed for the occurrence of virus. All leaf material will be destructed at harvest. The virus assays will be carried out in the institute Applied Plant Research in Lisse, The Netherlands. Transport of the transgenic plant material will be carried out according to the required regulations.

7. Succession (monitoring):

The field where the experiment has been carried out will be sprayed with Round-up to destruct remainders of plant material that might have been left over. In the seasons after the experiment, Buddleja, ornamental Malus, Hydrangea and roses will be cultured at the same place where the experiment has been done.
The field will be checked for several years for sprouted lily bulblets that might have escaped during culture or harvest. The chances that bulblets escape are negligible (see 6.3). All this material will be destructed by autoclaving.

8. Destruction of the transgenic plant material:

After harvest, the bulbs and their roots will be examined for the occurrence of virus. These assays will be carried out in Lisse, The Netherlands. Leaves and stems of the transgenic plants and of the nontransgenic control plants will be destructed by autoclaving.

9. Emergencies:

The experiment will be checked on a regular basis by Belgian and Dutch researchers. In exceptional cases, the plants may be effectively destructed by spraying with an appropriate herbicide.

10. Inspection:

The General Inspectorate of Raw Materials and Processed Products of the Ministry of Small Enterprises, Traders and Agriculture is responsible for the control of field trials with transgenic plants. To enable a proper control, the notifier is obliged to inform the appropriate authorities about the dates of planting and harvesting the material. In the field, the controllers make sure that the actions performed during planting and harvest are carried out in accordance with the ministerial permission and the various protocols. Furthermore, the controllers will take samples of the plant material for analysis in official laboratories.

11. Socio-economic aspects:

(see also paragraph 3).
There is no resistance against LSV in any lily genotype. Therefore, resistance cannot be introduced by crossing commercial genotypes with, e.g., wild genotypes collected in their natural habitat. LSV is spread by aphids and farmers now control viruses by spraying with mineral oils or synthetic pyrethroïds. It is of utmost importance for the environment and for public health to reduce the use of crop-protective chemicals. Virus-resistant lilies are important to enable sustainable cultivation of lily and LSV resistance can only be introduced in lily by the use of biotechnological methods.

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