

**Ministry of the Environment**

**Environmental Risks Department**

The Decision came into force on 8 April 2007.

Prague, March 6, 2007

Reference Number: 68730/ENV/06

## **D e c i s i o n**

The Ministry of the Environment of the Czech Republic as the administrative body competent according to § 5 of the Act No.78/2004 Coll., on the use of genetically modified organisms and genetic products as amended by the Act No. 346/2005 Coll. (hereinafter the „Act“) and in § 10 of the Act No. 500/2004 Coll., the Administration Code, as later amended,

### **has decided**

on the basis of an application of the Crop Research Institute, located in Drnovská 507, 161 06 Praha 6 – Ruzyně for granting consent for the deliberate release of genetically modified plum into the environment in the Czech Republic, pursuant to § 5 par. 8 of the Act:

**Crop Research Institute,  
Drnovska 507, 161 06 Praha 6 - Ruzyne**

### **i s g r a n t e d c o n s e n t**

**for the deliberate release of genetically modified plum trees c.v. Stanley clone C – 5  
with the inserted coat protein gene of Plum pox virus  
into the environment in the Czech Republic**

Requirements of the consent according to § 18 par. 6 of the Act:

#### **Authorised person**

Name: Crop Research Institute,  
Address: Drnovska 507, 161 06 Praha 6 – Ruzyna  
Identification Number: 00027006

## Specification of the genetically modified organism

Plum tree Stanley clone C-5 containing the coat protein gene of Plum pox virus.

## Specification of the genetic modification

Pl coat protein gene of plum pox virus (CP gene PPV) and two selective marker genes, i.e. (i) *nptII* gene from *E. coli* for neomycine phosphotransferase and (ii) *gus gene* from *E. coli* coding  $\beta$ -glukuronidase were inserted into the plum heritable material. *Agrobacterium tumefaciens*, the strain LBA4404 with binary vector pGA482GG/PPVCP-33 was used to transform a plum plant.

## Risk assessment results

So far, no adverse effects of genetically modified plum tree 'Stanley' clone C-5 with inserted Plum pox virus coat protein gene on the environment have been detected, although field trials with this GMO have been carried out in Europe for more than 10 years.

Plum is traditionally planted fruit species, which may run wild in the conditions of the Czech Republic. It is planted for direct consumption of fruits and/or their further processing. As regards allergenicity, "Pru d 3" allergen is stated in the List of Food Allergies for *Prunus domestica*. However, the effect of insertion of an insert on allergic properties of plum still has not been known, and it is not even a subject of the research in frame of this deliberate release into the environment. However, we cannot exclude any positive or negative change, and therefore this possibility must be taken into account.

The insertion of construct changes properties of recipient's organism so that plum obtains Plum pox virus coat protein gene into its genetic information. This gene encodes the synthesis of a pox virus coat protein. However, the pox virus coat protein is not produced in GM plum clone C-5. If there is any limited production of the pox virus coat protein in GM plum, it is a natural occurrence of viral product in a host genome, and therefore no harmful effect on human or animal health while they consume this fruit may be awaited.

*NptIII* and *gus* genes are used as selection markers. No harmful effects of these genes and their proteins on human, animals and plants have been found. The *nptIII* gene - neomycin phosphotransferase - confers resistance to kanamycin. If this gene escapes into the environment, only a recombination with bacteria pathogenic for human or livestock who are treated with this antibiotic can have a negative effect. Although, in agricultural practices this eventuality has not yet occurred anywhere all over the world within numerous field trials of GM plants with *nptIII* gene. Furthermore, the European Food Safety Authority (EFSA) has listed the *nptIII* gene to the group I according to its current spread in the environment and simultaneously according to the therapeutic significance of particular antibiotics. Group I is characterized by a minimal risk (resistance genes are considerably spread in the environment, irrespective of their presence in GMO, and antibiotics kanamycin and neomycin are not important with regard to their use in human medicine).

Risk of heteroencapsidation arises with the introduction of the insert, which can be caused by interaction of related viruses such as PPV and ZYMV, both belonging to *Potyvirus* species. As a result of heteroencapsidation during the interaction of PPV and ZYMV viruses in transgenic *Nicotiana benthamiana* expressing the PPV CP gene, the support of transmission of PPV-NAT isolate by the vector has been shown although this transmission normally does not occur. PPV is the only Potivirus occurring in *Prunus* species, and therefore this risk may be considered only as theoretical. Heteroencapsidation between viruses that are not related to PPV, occurring at *Prunus* species, such as PDV, PNRSV and ACLSV, is not known. The

synergic effect caused by the interaction of particular viruses with PPV could enable to overcome resistance of the transgenic plum trees. Research of that factor is the objective of current project. Synergic effects were investigated in frame of the EU project in INRA-Bordeaux. The results did not prove unambiguously the effect of synergic interaction of PPV with particular viruses on the transgenic plum trees C5. There is a potential risk of recombination of transgenic insert and virus. The results of the research in INRA-Bordeaux demonstrated that there is no occurrence of the combination of transgenic insert and PPV in C5. These results show practically zero biological risk related to the introduction of the insert. The plum with resistance to Plum pox virus is not more viable or competitive than non-transgenic plum tree.

A theoretical possibility of the gene transmission to *Prunus domestica*, *Prunus cerasifera* and *Prunus spinosa* species exists, which could thus obtain resistance to Plum pox virus. However, the Plum pox virus resistance will not have any effect in terms of natural choice and selection. An isolated location of the field trial was chosen in order to minimise possibility of an unintentional escape of the transgene via pollen dispersal.

The location of the experimental plot ensures both security against unauthorised persons and the space isolation from relative plant species.

If a scientific hypothesis on GM plum resistance to Plum pox virus is confirmed, the occurrence of this virus could be suppressed by reducing the number of its hosts. However, this effect would be expressed in a distant time horizon of one hundred or more years. Thus the Plum pox virus epidemic could be suppressed.

The output from the field trial will contribute to obtaining information about possible protection of plants against viral diseases, mainly Plum pox virus that is very important from the economical point of view.

### **Conditions for the use**

The genetically modified organism above mentioned shall be used only in the way described in the application submitted to the Ministry of the Environment (hereinafter “MoE”) on October 5, 2006, and supplemented with a submission delivered to MoE on December 21, 2006, and only when keeping all given conditions especially as follows:

- Every handling with the genetically modified material shall be under conditions minimising a possibility of transgene escape into the environment.
- To minimise possible spread of the transgene through seeds all the fruits shall be harvested even before getting ripe. Nevertheless, monitoring of the possible transgene escape into the environment shall be carried out by testing wild plants of plums, sloes and myrobalans.
- In laboratory analyses of plant samples (leaves, bark) – ELISA, RT-PCR tests – it must be taken into account that the taken samples have no ability to propagate or transmit the genetic material, and therefore every handling with them is not considered as the use of GMOs under § 3 par. 2 of the Act. However, fruits must be handled in a way preventing the possible spread of the transgene, for example a thermal inactivation thereof. Surplus parts of samples from an analysis shall be autoclaved, which is a standard process of handling with quarantine organisms in the Virology Departments of the Crop Research Institute despite a minimal possibility of spreading these organisms. Other plant remains that could be under certain conditions capable to propagate and transmit a heritable material, it means lignified sprouts that can be used as scions, shall be removed by their burning down directly at the field trial site, and the ash shall be put in the soil on the spot.

- The assessment of the field trial shall be carried out every year during a vegetation season from April to August. The assessment will proceed with the use of DAS-ELISA and RT-PCR methods. Laboratory tests shall be performed with three leaf samples.
- The plant material shall be transported in the contained space of a transportation vehicle under the supervision of a responsible person. All staff getting into touch with GM plums shall be trained by a professional consultant every year on rules for the use of GMOs. The samples could be transferred from the site of the field trial only for purpose of a laboratory analysis (see above). Laboratory analysis of taken GM plum samples shall be performed in laboratories of the Crop Research Institute (CRI), a phytotron, ground-floor, door No. 004B 005.
- According to the opinion of the Bee Research Institute it is necessary to keep the distance 1000 m between the transgenic plum trees and other plum trees in order to prevent the spread of transgene into the environment, which has been ensured. Moreover, at the flowering time no bee colonies can occur 700 m apart from transgenic plants, which has been controlled and met.
- After the field trial is finished, the transgenic trees shall be torn out and incinerated on the spot. The plot shall be deeply ploughed and monitored for possible occurrence of young shoots or plum saplings following two years. These pertinent young shoots shall be destroyed chemically with the total herbicide or mechanically.

#### **Other conditions on the use of GMOs under § 5 par. 10 of the Act**

The authorised person must whenever during the use of GMOs provide with samples of given genetically modified organism or its genetic material on request of the Ministry of the Environment or a laboratory according to § 28 par. 1 f) of the Act.

#### **Purpose of the release**

The aim of the research of an interaction of Plum pox virus, Plum stunt virus and Apple chlorotic leaf spot virus with transgenic plum plants of *Prunus domestica*, c.v. Stanley clone C – 5, which have been cultivated in an experimental orchard, is to find out the resistance stability of transgenic *Prunus domestica*, clone C-5 to PPV infection under field conditions. Simultaneously the interaction of heterologous viruses (PDV, ACLSV, PNRSV) with PPV and the effect of this interaction on the resistance of C-5 will be observed.

The experiment with the plum trees Stanley is a part of the research project NAZV MZe No. QDI 1360/2001/01 “Methods for the assessment of GM transgenic product effectiveness in plant protection; and the risk assessment of the implementation of these methods.” The project investigates benefits and risks of the use of GMOs in agriculture. The applicant is a project holder.

#### **Other requirements for labelling**

No special requirements in place, for deliberate release of GMO the common requirements for labelling of genetically modified organism have been laid down in law.

## **Place of the deliberate release into the environment**

Region: Praha, municipality: Praha 6 – Ruzyně, the airport area.

The field trial takes place on the plots belonging to the CRI and runs under the control of the CRI staff.

The GM plum trees have only been cultivated in Ruzyně 72970 cadastral territory, plot number 2795. The plot is a long strip located along the guidance system of the airport Praha - Ruzyně, and its total area is 21907 m<sup>2</sup>. The experiment is situated at the north corner of this plot. The field trial involves totally 52 trees planted in a planting space 2.6 x 3 m. Net area of the trial is 260 m<sup>2</sup>, the total area of the experimental location at the airport is 900 m<sup>2</sup> as there are further field trials with Plum pox virus beside GM plum trees at the location – apricot species with different level of resistance to PPV, which are infected by various strains of the virus. These field trials are planted around the GM plum trees, and therefore serve also as a buffer strip.

## **Requirements for monitoring and reporting of monitoring results**

During the whole vegetation season the trial shall be monitored and all non standard situations notified to the competent authorities, if occurred. Monitoring shall be performed next two years after the end of the field trial at the site of the experimental release into the environment as well as in the neighbourhood of planting areas including the non-specific monitoring aimed at the detection of possible unexpected effects of GMOs on the environment.

A prospective escape of the transgene into the environment shall be monitored once a month during the time of supposed growth of plum saplings, i.e. from June to August. Monitoring shall be carried out up to 1 km from the field trial location, where relative plants with the potential for outcrossing can occur. The distance can be extended, if it is considered as suitable measure with regard to terrain conditions. One-year saplings of wild plums, myrobalans and sloes will be looking for, and analyses will be performed continuously during a vegetation season. The presence of *gus* gene shall be tested. RT-PCR with specific primers for PPV-D shall be used for the transgene presence analyses.. Monitoring shall be performed by the staff responsible for the GM plum project and trained by the professional consultant on handling with genetically modified organisms.

## **Validity**

### **2007 - 2010**

The plum is a multi-annual plantation, thus the observation must be repeated in several vegetation seasons in order to carry out the right assessment of the field trial. This Consent shall apply for a period from **2007** to **2010**. The end of the field trial is supposed on 31.12. 2010, while this period may be prolonged until the information and required data are obtained, i.e. the interaction of the genetic modification and the infection by one and several viruses.

## **I n s t r u c t i o n s**

Within 15 days from the date of the notification of this Decision there is an opportunity by the submission to the Ministry of the Environment, Vršovická 65, 100 10, Praha 10, to make representation to this Decision according to § 152, par. 1 of the Act No. 500/2004 Coll., on administrative proceedings (Administrative Code), whereupon the Minister for the Environment will decide.

Ing. Pavel Forint  
Department Director

This Decision shall be received by:

- A. Participant in the proceedings for personal delivery:  
Crop Research Institute, located in Drnovská 507, 161 06 Praha 6 – Ruzyně
  
- B. For information:
  - 1. Ministry of Health
  - 2. Ministry of Agriculture
  - 3. Praha 6 District Authority, Čs. Armády 23, 160 52 Praha 6
  - 4. Prague City Hall, Department of Crisis Management, Platněřská 19/111, 110 00 Praha 1