Notification report

General information

Notification Number: B/BE/03/V1

Member State:*Belgium*

Date of Acknowledgement:*23/12/2002*

Title of the Project:
A field assessment of the introduction of the self-compatibility trait in transgenic Elstar trees; flower bud formation, fruit set, yields, production efficiency and fruit quality.

Proposed period of release From:*01/03/2003* **To:***01/11/2006*

Name of the Institute(s) or Company(ies): *Katholieke Universiteit Leuven, Fruitteeltcentrum, Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen*;

3. Is the same GMPt release planned elsewhere in the Community?
No

4 - Has the same GMPt been notified elsewhere by the same notifier?
No

Genetically modified plant

1. Complete name of the recipient or parental plant(s)

Common Name	Family Name	Genus	Species	Subspecies	Cultivar/breeding line
apple	*rosaceae*	*malus*	*malus domestica*		*Elstar*

2. Description of the traits and characteristics which have been introduced or modified, including marker genes and previous modifications:
Elstar apple trees have been modified in their ability to self-fertilise and thus to produce fruit following self-pollination. Transgenic Elstar plants were created using antisense and sense constructs of an S-Ribonuclease (S-RNAse) allele, originally cloned from the apple variety Golden Delicious. The S3-cDNA encodes for one of a family small glycoproteins, the S-RNAses. The S-RNAses are involved in the self-incompatibility reaction of Malus sp. and are responsible for the breakdown of ribosomal RNA in the pollen tubes of self-pollen i.e. pollen which contain an S-allele identical to one of the female parents alleles. Antisense expression of the truncated 3' or 5' terminus of this allele, or sense expression of the full-length cDNA of this allele results in partial or complete inhibition of this regulatory mechanism. The S-RNases are naturally only expressed in the carpels of the flowers.
The transgenic Elstar plants also contain the nptII marker gene, which is used for the identification of plant (cells) containing the T-DNA insert(s). nptII codes for the gene neomycin phosphotransferase, which confers resistance to the antibiotics kanamycin, geneticin and neomycin.

Genetic modification

3. Type of genetic modification:
Insertion;

4. In case of insertion of genetic material, give the source and intended function of each constituent fragment of the region to be inserted:
The origin, description function and size of the individual components of the DNA inserts is summarized in table1.

Table 1 functions, origins and size of genetic elements contained in transformation vectors

Gene Functions and Origins of the T-DNA: Insert, Source, Function, size/position

RB:
Right border Ti-Plasmid Agrobacterium tumefaciens.
Transfer of insert to plant. One of 2 repeat sequences flanking the T-region of A. tumfacians Ti plasmids. These borders are essential for the excision of the T-strand after contact of the bacterium with wounded plant cells.
0.147 kb

35S-prom:
Cauliflower Mosaic Virus (CaMV) promoter region.
Promoter: For initiation of transcription, drives constitutive expression of transgene, and contains double enhancer for stronger expression.
0.8 kb

S3-cDNA 0.717 kb
3' end of S3-cDNA 0.333 kb
5' end of S3-cDNA 0.384 kb
Malus x Domestica Borkh. cultivar Golden Delicious.
Endogenous Malus Elstar cDNA S-allele encoding for S-RNAse involved in the self-incompatibility response. EMBL accession no. U12200

35S-ter:
Cauliflower mosaic Virus (CaMV) terminator region.
Terminator: stop transcription of transgene.
0.3 kb

Nos-prom:
Nopaline synthase gene from the Ti plasmid of Agrobacterium tumefaciens.
Promoter: initiation of transcription.
0.307 kb

nptII:

Transposon Tn5 from Escherichia coli.
Confers resistence to aminoglycoside antibiotics such as neomycin, kanamycin and geneticin.
1.2kb

Ag7-ter:
Terminator region of Ag7 gene from the Ti plasmid of Agrobacterium tumefaciens.
Terminator: stop transcription of Ag7 gene.
0.3 kb

LB:
Left border Ti-Plasmid Agrobacterium tumefaciens.
Transfer of insert into plant.
0.161 kb

6. Brief description of the method used for the genetic modification:
Insertion of genetic material into the host genome (transformation) was carried out by via co-cultivation with the kanamycin-sensitive Agrobacterium tumefaciens strain EHA105 (Hood et al., 1993). For each construct 1500 leaves of in vitro-cultivated apple cultivar Elstar, were infected with A. tumefaciens, essentially as described by De Bondt et al. (1996). The leaves used for transformation were the youngest four, fully expanded leaves of 5-week old micro-propagated shoots.

7. If the recipient or parental plant is a forest tree species, describe ways and extent of dissemination and specific factors affecting dissemination:
Both the recipient and parental plants are apple species. Dissemination can occur either by pollen transfer or by seed dispersal. However in commercial orchards, dissemination occurs by man-made vegetative propagation of elite lines by grafting onto commercial rootstocks. The majority of commercial apple cultivars are self-incompatible, meaning that they require pollen from a different, compatible parent for successful fertilization and fruit set. The efficiency of pollen transfer is strongly influenced by environmental conditions, such as temperature, rain, cloud, wind etc., due to the impact of these factors on insect (honey-bee activity). Insects (honey bees) are the most important mechanism of pollen dispersal transfer, and for this reason commercial orchards often contain beehives. A small amount of wind-pollination also takes place, but studies indicate that this is only effective in achieving cross-pollination within a radius of 5 - 10m around the pollen source (Wertheim, 1991). Our own studies indicate that wind-pollination is responsible for a maximum of 10% of the fruit set (Porta, 1996), when the pollinator source is within a radius of 5m.
Seed dispersal naturally takes place through animal feeding (primarily small mammals and birds), but this activity is far exceeded by man-made activities, such as the production and transport of apples and the discarding of apple cores, containing seed by consumers. Despite this, dissemination by seed dispersal and germination is a very inefficient strategy in apple. This is demonstrated by the fact that although thousands of apples are allowed to lie on the ground in commercial orchards, essentially no wild or escape seedlings are found either in or around these orchards. This is because of the need of apple seeds for a period of stratification (approx. 3 months at a 2 - 4oC), for germination and their relatively slow growth. Dispersion via seeds is also limited by the large amount of space required by the developing seed and the long juvenile period of apple (the tree will only begin flowering when it is 6 - 7 years old)

Experimental Release

1. Purpose of the release:
European apple and hard-fruit production is characterized by large annual fluctuations in yield, partly as a result of variations in fertilisation-efficiency during the flowering season. These variations are directly and indirectly the result of the self-incompatibility (SI) trait, which requires that flowers be cross-pollinated with pollen from a compatible cultivar. Cross-pollination itself is primarily insect-driven, although wind-pollination may also occur. Both processes are strongly influenced by the weather conditions (rain, temperature, cloud, wind speed, etc.) prevailing during the flowering period. To try and reduce this yield variability, we have developed transgenic apple lines in which the expression of the gene responsible for the self-incompatibility phenotype has been suppressed, and we now have transgenic trees displaying differing degrees of self-compatibility (self-fertilisation). These self-fertilising trees form an important and unique tool to study the potential advantages and disadvantages of self-compatibility to European fruit production, and for the first time allow us to study the impact of the introduction of a single trait against a constant genetic background in apple.
The aim of this trial is to determine the impact of self-compatibility on a variety of commercially relevant agronomic characteristics, including fruit set, total yields, biennial bearing and fruit quality. The results will help determine the relationship between fruit set, quality and yield, and on the other hand the requirements for thinning (chemical thinning or self-thinning varieties) under both favorable and unfavorable weather conditions. This represents a logical extension of our previous analyses of these lines under controlled greenhouse conditions.
It is not the intention to develop transgenic, self-compatible Malus varieties for commercial release. Rather, the results of the field trial will allow a thorough quantitative and qualitative assessment of the potential contribution of this single characteristic to modern production programs. We anticipate that the correct level of self-compatibility can decrease the dependency on cross pollination, bees, and good weather during flowering, and thereby lead increased yield efficiency and greater returns. This know-how can be used in classical breeding activities to focus on the introduction of the correct biological traits, to improve the cropping efficiency of new cultivars.

2. Geographical location of the site:
The field trial will be located on a 20 ha orchard site owned and managed by the Fruitteeltcentrum, KUL, localized at Fieldstation Rillaar, Steenberg 36 3202 Rillaar, situated on the cadastral map of Aarschot in the 5th division section D nrs. 345b, 347x_, 348x, 451d, 451e, 452a, 453a, 455, 456b, 456c, 458f, 458f2, 458g, 458h, 458m2, 458p2, 458s2, 458z, 484. Excepting the nrs. 458m2, 458p2 and 458s2, the entire area is owned by the KUL. The Fieldstation is managed by Ir. Johan Verheyen, and securely fenced in with entry restrictions for non-university personnel. We consider it important that this test site should be located on a land area owned and managed by the KUL to ensure correct and proper monitoring of the site, as well as to ensure proper waste management.
The test plot is located on site #458 M2

3. Size of the site (m2):
160 trees will be contained in 2 covered tunnels, 46m long, with a total area of 713 m2. The tunnels are additionally surrounded by a 4m high windbreak at a distance of 2.5 m2 from the tunnel sides, and 6m from the tunnel ends, giving a total area of 1189 m2.

4. Relevant data regarding previous releases carried out with the same GM-plant, if any, specifically related to the potential environmental and human health impacts from the release:
Not applicable

Environmental Impact and Risk Management

Summary of the potential environmental impact from the release of the GMPts:
Self-compatible GMHPs can theoretically become more persistent than the parent plant because as a fully-or partially self-compatible variety it is no longer dependent on cross-compatible varieties to produce viable seed. Therefore for individual trees in isolation (i.e. under conditions where there are no cross-compatible varieties within pollination range, or when environmental conditions are sub-optimal for wind- or insect- mediated pollination), then the transgenic apple trees would theoretically be able to survive longer than the non-transformed parent by producing viable seed. However this will not provide a selective advantage for better survival because such inbreeding in Elstar (and other varieties), leads to a loss of viability in the progeny i.e. inbreeding depression. This inbreeding depression is characterized by reduced growth rates, loss of fertility and poor fruit quality (unpublished data), and is therefore actually a progressive, selective disadvantage.
There is no selective advantage to plants expressing the nptII marker gene, which is already naturally present in bacteria found in the biotope.

Brief description of any measures taken for the management of risks:
The test plot will be maintained at a minimum 100m distance from the nearest sexually compatible plant species. The trees will be housed in 2 x 46m long tunnels, covered the entire year round with netting to exclude pollinator insects. The tunnels are protected along the sides and the ends with 1.8m high plastic sheeting. 1 week before the flowering period, the tunnels will be covered with an additional pollen-proof netting, to prevent the escape of any transgenic pollen. This netting will be kept in place until one week after flowering. It is also important for the scientific results of this experiment that no cross-contamination of pollen occurs. The entire plot will further be surrounded by a 4m high windbreak of nylon netting to reduce wind speeds by 70% and to minimize the possibility of wind-mediated pollen transfer events.
The trees will be monitored every week during the growing season for the possibility of a secondary flowering, in which case the flowers will be removed and destroyed. To prevent seed dispersal, all fruit that has fallen to the ground will be collected throughout the year and destroyed by composting down on site. At harvest, all fruit will be handpicked and collected either for laboratory analysis (seed number, weight, quality etc.), and subsequent incineration, or direct incineration. All seeds will be collected separately and destroyed by soaking in sulphuric acid. The personnel involved in this trial have experience in the handling and processing of transgenic plant material from previous experiments.
Therefore there is a 3-fold restriction in access to the test site. Firstly the site is maintained on the breeding station itself, where access is restricted to personnel and registered visitors. The test plot is then surrounded by a 4m high wind break, with access via a door, and finally the plants are housed inside tunnels which are accessed by a locked, 2m high door at one end of each of the tunnels

Summary of foreseen field trial studies focused to gain new data on environmental and human health impact from the release:
Not applicable.