

The Decision came into force on 22 June 2016

Prague, 2 June 2016
Reference Number 23920/ENV/16

D e c i s i o n

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

has decided

pursuant to § 5 par. 8 of the Act on the basis of an application of the Institute of Experimental Botany AS CR, located in Rozvojová 263, 165 02 Praha 6, on granting consent for the deliberate release into the environment of genetically modified soya with gene LTB (2016-2021), as follows:

Institute of Experimental Botany

Rozvojová 263, 165 02 Praha 6 – Lysolaje,

is granted consent

for the deliberate release into the environment of
genetically modified soya with the LTB gene (2016-2021)

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

Authorised person

Name: Institute of Experimental Botany AS CR
Address: Rozvojová 263, 165 02 Praha 6 - Lysolaje
Identification Number: 613 89 030

Specification of the genetically modified organism

Soya line No. 157 was modified with the DNA fragment coding for hygromycin phosphotransferase bacterial enzyme. This enzyme confers plant resistance to hygromycin. In addition, the LTB gene (i.e. the gene for non-toxic beta subunit of temperature labile enteric toxin originating from *Escherichia coli* H10407) is expressed in a plant under the control of glycinine promoter and terminator for the LTB gene regulation. LTB gene expression occurs only in plant seed.

Specification of the genetic modification

GM plants have been developed by the insertion of pGly:ER-LTB plasmid into the genome of 'Jack' variety. Biolistically inserted DNA fragment contains pGly (glycinine gene promoter), fragment of LTB coding for the *E. coli* protein (monomer of beta-subunit), t-Gly (polyadenyl signal for glycinine gene), pUbi3 (potato gene promoter for ubiquitin), hpt (selection gene coding for hygromycin phosphotransferase), and tUbi3 (ubiquitin terminator from the potato gene).

Vector containing pGly:ER-LTB insert was prepared and used at the Plant Genetic Research Unit, USDA Agricultural Research Service, Donald Danforth Plant Science Center, Montana, USA. DNA fragment of length ca 5 kb that was segregated from the vector was introduced into soya embryo using biolistic.

LTB gene (gene coding for beta subunit of enteric toxin from *Escherichia coli* H10407) was prepared as a synthetic gene with codons adapted to potato translational apparatus.

C-end of the synthetic gene is connected with the FLAG (DYKDDDDK) epitope facilitating its immunodetection. The FLAG epitope is followed by the KDEL signal in order to maintain the protein in the endoplasmic reticulum. The gene product then produces pentamers that enable linking of the whole toxin to the animal intestinal epithelium.

Above mentioned bacteria was not used either at the mentioned Montana workplace. Hpt gene integrated under the regulating sequences from potato was obtained from another laboratory in the form of plasmid and it was not prepared for soya transformation purposes.

Inserted DNA is located in the plant nuclear DNA, one locus on the haploid set of chromosomes. It is highly probable that the integration place is in the nuclear DNA as it resulted from the segregation founded in seeds harvested from a T1 plant.

LTB transgene is expressed from the pGly promoter, which is not operational in chloroplasts. Remaining ca 650 base pair represent sequences of vectors from which individual segments were originally cloned.

Whereas hpt gene coding for resistance to hygromycin is expressed in all parts of the plant, the LTB gene is expressed only in soya seeds.

The FLAG epitope, which is a part of the LTB protein, was repeatedly detected in the soya line No. 157.

After transformation all plants were selected at the cultivating medium containing hygromycin antibiotics, and we expect that inserted hpt gene was expressed in the GM plant.

The LTB protein was observed in soluble protein extracts of seeds by the epitope presence using the relevant antibody.

Risk assessment results

Risk assessment has been carried out according to the Act. The principles of good experimental practice (GEP) and good agricultural practice will be applied on genetically modified soya cultivation. Field trials with the soya line No.157 derived from 'Jack' variety will be carried out in order to obtain the expression characteristics of transgene being expressed mainly in seed.

Soya is considered relatively safe plant species, which spreading can be controlled as no weedy compatible species occur in Europe and therefore weedy cross hybrids are not produced. Soya belongs to fam. Leguminosae, and it is cultivated in more than 35 countries around the world. Soya is considered the oldest crop originated in the North and Central China. It is an annual, low-temperature sensitive herbaceous plant that does not survive winter conditions. That is why the risk to the environment is very low. In this case, the risk for the deliberate release into environment consists in the presence of active selective genes. In addition, the tested genetically modified line will not be used as food or feed, and thus the entry of inserted genes into the food chains is minimal. Risk level depends on correct manipulation with GM plants. For that purpose the Emergency Plan and the Operational Rules are elaborated and all the staff handling with GMO shall become acquainted with it.

In the Institute of Experimental Botany AS CR, soya (*Glycine max* L., Merr.) is used as a recipient. At this workplace, the first transgenic soya material was handled in line with the notification Ref. No. 9688/ENV/07 issued on February 2007.

The use of identical genetically modified soya under the contained use regime in the Institute of Experimental Botany workplace (address Na Karlovce 1a, Prague 6) was initiated on the basis of the notification Ref. No. 25136/ENV/09 issued on 3 April 2009.

LTB is the tested gene, which is expressed in plant seed and could probably play a role in vaccination as an adjuvant. It is assumed that the presence of beta-subunit bacterial protein does not cause an adverse effect on human or animal health. Insertion of construct may induce adverse insertion mutations. The line No. 157 was chosen according to the best growth characteristics and the production of transgenic protein; therefore plants with undesirable traits were excluded.

The line No. 157 contains hpt gene coding for the plant resistance to hygromycin antibiotics that is used for selection of transformed soya plants. According to the Opinion of the European Food Safety Authority (Opinion, 2004), hpt gene belongs to the group of genes having no adverse effect on human or animal health or the environment. No data about its harmfulness is known. No selective advantage resulted from the hpt gene insertion exists in nature as there is no selection pressure giving some advantage to its carrier. Given system is possible to be used only for in vitro selections. There is no other selective advantage for soya plants coming from above mentioned genetic manipulation.

Seed proteins from the line No. 157 were compared with the proteins from the parental 'Jack' variety using 2D electrophoresis, and no significant changes in the amount of main seed proteins incl. glycinine and conglycinine were recorded. New LTB protein was detected in the soya line No. 157.

Seeds are the only part of plants useable for human alimentation. They are commonly further processed (soya milk, tofu, flour etc.) before consumption.

Genetic modification of soya that results in the accumulation of bacterial protein beta-subunit pentamers (up to 2.4 % of proteins in the seed) does not change biological characteristics of the plant.

Protein for beta-subunit itself is not toxic to human. What is more, toxic effect of hygromycin phosphotransferase, the product of the hpt selective gene, has not been proved as well.

Although the soya produces 15 proteins having the reaction with IgE from individuals being allergic to soya products, the soya varieties without a main soya antigen have already been found (Gly mBd30k corresponds to the protease cysteine).

This GM soya is not intended for feed. What is more, the genes used in the modification were not proved to have any harmful effects on animal health or food chains.

Biological characteristics of soya in the current geographical latitude significantly limit possible invasion to the environment, and it is not expected that these characteristics could be influenced by the given genetic modification.

Soya does not produce weedy plants. In North America, no soya plants were found outside the field. It is not expected that the performed genetic modification will change either growth characteristics of plants or interactions with the environment.

Soya plants are almost fully self-fertile and soya is not cultivated in the nearest vicinity of the plot.

In addition, the GMO is not expected to be spread outside the intended plot (small area of the plot and the low number of planted individuals facilitate the control of spreading, if appropriate).

GMO field trials will be carried out on a grassed plot in the fenced area of the Academy of Sciences in Lysolaje (complete area is guarded by security services). Given plot will be used repeatedly for the field trials. Spontaneous spreading of transgenic plants can be practically excluded.

Due to the type of flower formation and flowering process a pollination happens before flower opening, and therefore cross-pollination happens less than in one per cent of cases.

Neither seeds nor fruits are attractive for animals and are not spread by them. Seeds have very low dormancy potential, and thus soya can grow as weedy plant on the plot in the following year after the harvest only under specific conditions. Weedy plants are easy to recognise in the following crop vegetation and can be disposed mechanically.

Effect on non-target organisms comes on force only in relationship of plants with specific modifications having an effect on pests, particularly insect. Genetically modified soya deliberately released into the environment does not contain such modifications. Change in the protein composition proceeds only in seeds and only change in plant attractiveness for common pest can be proved. With regard the fact that the soya does not originate in the Czech Republic and is not cultivated widely here the pest occurrence is rather sporadic.

Rarely, soya can be damaged by animal pest like nematode (however, 'Jack' variety is resistant to soya cyst nematode *Heterodera glycines* that devastates soya vegetation in the United States from the mid of last century, spider mites (particularly fam. Tetranychyidae in our country), aphids, sage leafhoppers, Heteroptera, and Elateridae. Evaluation of GMO effect on these pests will not be carried out in the course of the field trials.

Risk assessment results

No adverse effect of GM soya on human and animal health or the environment is expected under the conditions defined in this Decision.

Conditions for the Use

The above mentioned genetically modified organisms shall be used only in the way described in the application Ref. No. 23920/ENV/16 submitted to the Ministry of the Environment on April 6, 2016, and supplemented with the submission to the Ministry of the Environment on May 20, 2016, provided that all given conditions have been met, particularly as follows:

- All handling with the genetically modified material shall be under conditions minimizing a possibility of transgene escape into the environment.
- The principles of good agricultural practice shall be kept and the principles of good experimental practice shall be applied.
- GM soya shall be handled only in line with binding procedures, and must not be delivered to any persons who are not trained about the rules for use of GMOs.
- The field trials shall be carried out by staff trained for the use of GMO at the experienced workplace specialized for GMO trials, according to the unified methodology. Trials shall be conducted under the supervision of the professional consultant.
- The staff that will get into contact with seed or green plants when assessing or harvesting them shall be trained about rules for the use of genetically modified organisms, shall be equipped with protective tools and warned of the prohibition of consumption and use as feed of any type of the experimental material. The staff shall become acquainted with the Emergency Plan and the Operational Rules to be able to solve problems, if appropriate, and shall be informed on their duty to report on any health problem that can relate to the deliberate release to the environment. The written records shall be kept on all handling with the experimental material.
- The trained staff shall ensure the preparation of the plot before setting up the field trial as well as the agro-technical measures concerning standard treatment of the spring barley. They shall carry out ancillary works in connection with the setting up of the field trial, sampling, assessments and manual harvesting in the presence of the professional consultant.
- GM plants pre-cultivated in a cultivation room (higher temperature and a short-day) will be prepared for the cultivation season 2016. Plants will represent generations T7 and T13, respectively. This procedure will be applied also in the

next vegetation seasons. The number of plants released to the environment may vary every year depending on results of the glasshouse experiments and field trials.

- The plot is located in the fenced area of the Academy of Sciences. During the period approved in the Decision the trial shall be conducted at the same plot and properly labelled with warning signs
- Grass and original soil shall be removed from the plot before GM soya planting, then digging shall be performed, a mixed potting soil (brown soil and pearlite) shall be brought and the basic NPK fertilisation shall be applied.
- Following procedures will be applied: planting and plant lashing, watering, fertilisation, biological protection application, chemical treatment commonly used for the Institute's greenhouses. The plot will be irrigated.
- No buffer zone will be set around the trial plot. The trial shall be located in the part of grassed plot neighbouring the B4 greenhouse. No crops are cultivated in the vicinity, only a built-up area occurs nearby.
- Plant seeds intended for the release into the environment shall be stored in the refrigerator in the B2 building, the Institute of Experimental Botany ASCR. Seeds will be sprouted, plants put in the pots and pre-cultivated in the cultivating room and the B4 greenhouse (a workplace is secured from the entry of non-authorised persons).
- Control plants intended for the next vegetation season shall be obtained from seeds harvested from plants being isolated in the greenhouse. The amount of produced sub-unit protein per seeds weight shall be analysed using the Western blot and the antiFLAG specific antibody.
- With regard to soya plant biology and small size of the trial, particularly correct outplanting of GM soya plants and their labelling on the plot shall be controlled.
- GM plants of required size pre-cultivated in the cultivation room and the greenhouse shall be taken out on the plot in closed containers labelled "GMO" and planted out manually. All plants shall be planted out on the plot, and the control of completeness in line with the list shall be done.
- At least two-line distance shall be kept between lines with transgenic plants and 'Jack' original variety serving as control plants.
- With regard to the limited space to be used it is expected that totally 50 GM plants of the line No. 157 and control plants shall be released into the environment in the vegetation season 2016.
- It is not expected that the number of released GM plants will increase as the capacity of rooms suitable for plants pre-cultivation is limited.

- Plants may be planted in density 9 – 12 individuals / m²; it is approximately 10 individuals / m² after planting out.
- The total planned area of the trial with GM soya deliberately released into the environment shall be ca 10 m².
- Plants on the plot shall be bound to a supporting bean-pole, and a protective net against pest shall be fasten onto them and protect the whole field. Plants will be irrigated and fertilized. In case insect pest occurrence (spider mites and thrips), biological protection using predaceous mites (*Phytoseiulus permisilis* and *Amblyseius cucumeris*) shall be applied. Occurrence of diseases is not expected.
- The trial plot with the GM soya shall be secured from the entry of non-authorized persons during vegetation season using a spatial separation and labelled with two visible warning signs „GMO – NO ENTRY”.
- The trial plot with the GM soya shall be secured from animals using a fence around the area of the Academy of Sciences and an upper net placed above cultivated plants. Neither pods nor seeds are attractive to animals and therefore no risks for animal health are in place.
- Harvested GM soya shall not enter the food chain or feed chain. Harvest shall be realized by trained staff of the Institute of Experimental Botany ASCR, under the supervision of a professional consultant.
- Harvesting shall be carried out manually plant by plant. All plants shall be harvested simultaneously. Harvesting shall be performed repeatedly according to the gradual pods maturation.
- Every plant shall be harvested individually and pods shall be put into paper sacks, clearly labelled with codes so as to prevent their replacement, and ensured against the material loss; these sacks shall be put into the labelled lockable plastic container.
- Containers labelled with the text “Genetically modified organism – not for food or feed” shall be transported to the B2 building, and pods shall be left to dry by air in a lockable room.
- GM soya pods shall be transported separately from control plants pods. Before their storage the hulled seeds shall be dried by air and stored separately in the contained labelled boxes in the refrigerator.
- Required number of hulled seeds shall be homogenized in the laboratory and a soluble protein shall be extracted. Individual samples shall be prepared from three seeds originated from the same plant. Seeds from 5 – 8 individual plants shall be used for the analysis. The remaining seeds shall be stored in refrigerators for further plant cultivation except of ‘Jack’ non-transgenic variety.

- Seeds from Jack control variety shall be harvested as well, and used for the protein content analysis. However, they shall not be planted out repeatedly in the next vegetation season in order to avoid possible outcrossing of these plants.
- Measures to decrease or prevent pollen/seeds escape are not necessary to take. Although the outcrossing between 'Jack' control variety plants and plants from the line No.157 may happen, harvested seeds from control plants shall not be used for the pre-cultivation of new control plant following years.
- With regard the fact that no wild sexually compatible species occur in the Czech Republic, and soya is not cultivated near the Institute area, no measures will be taken.
- Plants and their remains shall be burnt on the plot after their partial desiccation. Remaining unused seeds, incl. those from 'Jack' control variety shall be inactivated using an autoclave.
- Written records on handling with the harvested experimental material as well as the waste shall be kept.
- Guidelines shall be changed according to the source material and the goals of the trials, and shall be amended regularly. Maps of the trial shall be integral part of the documentation at the Virology department, and shall be submitted to the Ministry of the Environment.
- Written records shall be kept on all handling with the GM material incl. transport and shall be also a part of the separate field Diary.
- An accident may happen during transportation of plants or pods on / from the plot (for example due to person's falling down); in such the plant material case may drop out on the lawn or flagstone. The material can be easily picked up and further used. If the original package is damaged and seeds are dropped out, the place of accident would be decontaminated immediately by manual collecting into the new package.
- If necessary, the trial is possible to treat with a non-selective herbicide (e.g. Roundup), its effectiveness shall be verified and the occurrence of escaped transgenic plants on the plot shall be monitored the next year. The procedure can be repeated. The herbicide shall be stored together with plant protection chemicals in the B3 greenhouse neighbouring with B2 building.
- During the storage of seeds in the B2 building fire may be marginally expected. GMOs affected by fire can be considered liquidated. If plant material is possible to save, transgenic plants and harvested seeds shall be saved first in order to prevent uncontrolled contamination of the building with GMOs. Director or the professional consultant shall decide about their further use. In other points, fire shall be eliminated in line with the Fire and Emergency Plan.

- Institute of the Experimental Botany ASCR shall in accordance with § 19 letter c) of the Act submit to the Ministry data on the amount of GM soya and on handling with it in written and electronic form annually, always by 15 February in the calendar year, and pursuant to § 19 letter d) of the Act submit within 60 days from termination of the use of genetically modified organisms a final report on the course and consequences of this activity, particularly with regard to any risk for human health and the environment. The final report shall be submitted in English as well pursuant to the Annex to the Decision of the European Commission 2003/701/EC.

Other conditions stipulated under § 5 par. 10 of the Act

- Every year no later than 30 days after the sowing of GM soya, the Institute of Experimental Botany ASCR shall provide to the Ministry of the Environment written information on the deliberate release into the environment and on the area of GM soya, and the map with the precise location of the trial incl. GPS lines as well as the map of the trial.
- The Institute of Experimental Botany must on request of the Ministry of the Environment or the laboratory according to § 28 par. 1 letter f) of the Act provide samples of the tested GM soya or its genetic material at any time in the course of its use.
- The Institute of Experimental Botany ASCR must according to § 28 and § 31 - 33 of the Act cooperate with the authorities (the Ministry of the Environment, the Czech Environmental Inspectorate, the Central Institute for Supervising and Testing in Agriculture) during the control of plots, premises and facilities intended for the use of GMOs, or the plots, premises and facilities in which GMO may be used, including written records, at any time in the course of its use.
- The Institute of Experimental Botany ASCR must on request of the Ministry of the Environment or the laboratory according to § 28 par. 1 letter f) of the Act provide samples of the tested GM soya or its genetic material at any time in the course of its use.

Purpose of the release

- The purpose of the deliberate release of the GM soya into the environment is to verify the stability of LTB transgene expression under the field conditions, while the common yield has been kept. The non-transgenic 'Jack' variety shall be also planted out as control plants.
- Field trials with the transgenic soya line No. 157 derived from 'Jack' variety are carried out to obtain the characteristics of transgene expression that is performed particularly in seeds. In addition, above mentioned field trials are carried out to

verify total seed production, the stability of transgene expression and protein content in variable environment *in campo*. The aim of the release into the environment is to specify the amount (and seasonality?) of the specific protein production. The assessment of sub-unit protein production coded by the LTB gene shall be performed using the Western blot and the antiFLAG commercial specific antibody. Plant health shall be also assessed.

- 'Jack' variety belongs to the soya maturity group II (seeds become mature later) and it is adapted to the geographical latitude from 40 to 42 degrees. Information on how do climatic conditions in the Czech Republic suit to this particular variety and whether they do not have negative effect on protein content in seeds (in particular protein coded by the inserted transgene) will be the result of the trial.
- Expected result of the release into the environment is the assessment of seeds production and the portion of transgene protein in seeds under the conditions outside greenhouse or cultivation room.

Other conditions for labelling

General conditions for labelling of GMO in compliance with the law are applied.

Containers that will contain seeds or parts of GM soya plants with the LTB gene shall be labeled as follows: "Not for food or feed! Delivery to non-authorized persons prohibited!"

The unique identifier pursuant to Commission Regulation (EC) No. 65/2004 for GM soya plants with the LTB gene has not been defined yet.

Location of the deliberate release into the environment

The Institute of the Experimental Botany ASCR will realise the field trials in the fenced area of the Institutes ASCR located in Rozvojová 263, 165 02 Praha 6 Lysolaje. The plot where the release into the environment will be performed is the property of the Institute of the Experimental Botany ASCR. The trial plot is located on the North side of the B4 and B2 building, next to the building where all other necessary equipment for handling GMO is stored.

Region: Praha

District: Praha 6 – Lysolaje

Plot name: Area of the Academy of Sciences Czech Republic

Name of cadastral territory and parcel No.: Cadastral area Lysolaje, parcel No. 513/172

Parcel size: 2246 m²

Coordinates of the 10 m² trial plot: 50°07'39.0"N 14°22'56.3"E

The plots incl. parcel numbers used in the following years shall be notified to the Ministry of the Environment at least 30 days before expected sowing.

Requirements for monitoring and reporting of monitoring results

The monitoring plan has been based on the results of risk assessment, and its objective is to early observe and identify both expected and unexpected effects of GM soya plants with the LTB gene on the environment after their deliberate release into the environment. Concerning the low frost-resistance of soya as well as non-existing dormancy, there is no risk of escape of transgenic plants into the environment.

Monitoring shall be carried out during the course of the field trials and after the termination thereof. The trained staff of the Institute of Experimental Botany ASCR with the professional consultant shall carry out monitoring.

The trial area shall be always properly checked for the presence of any germinating plants before planting GM soya plants, minimally twice in the beginning of the vegetation season. The area shall be treated with the non-selective herbicide (e.g. Roundup) before setting a new trial. It is not necessary to monitor other areas.

Field trials shall be controlled once a week starting from the planting out the GM plants. Date control shall be recorded in the Field diary. Seeds stored in the refrigerator in the B2 building shall be controlled at random.

Identical trial area shall be used for repeating the trial next vegetation season (after fertilising the soil), because plants germination cannot be expected in light of soya low-temperature sensitivity. If such unlikely phenomenon happened, these plants would be manually eliminated from the area before next planting.

In the subsequent year after the trial termination the experimental site shall be checked in order to monitor the occurrence of soya plants, if any.

Any harmful effects on the environment resulting from the deliberately release of GM soya plants into the environment shall be notified immediately to the Ministry and other relevant competent authorities.

In case of an accidental escape of GM plant material into the environment during its transportation, the affected place shall be managed in the same way as the experimental plot. The monitoring in such place and its vicinity shall be carried out in the same extent as on the experimental site, including yearly observation in the subsequent year.

Every year the monitoring report summarising the results of observations during the field trial will be submitted to relevant competent authorities.

After the end of monitoring, the written report on its course and results shall be submitted to the Ministry of the Environment according to the Act and the the European Commission Decision 2003/701/EC.

Validity

The consent shall apply to December 31, 2021.

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 to make representations to this Decision by the submission to the Ministry of the Environment, Vršovická 65, 100 10 Praha 10 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. Karel Bláha, CSc.
Department Director

This Decision is addressed to:

A. Participant in the proceedings for personal delivery:

The Institute of Experimental Botany ASCR, Rozvojová 263, 165 02 Praha 6 –
Lysolaje

B. For information:

1. Ministry of Health
2. Ministry of Agriculture
3. Prague City Council