FORMAT FOR THE PRESENTATION OF THE RESULT OF DELIBERATE RELEASE INTO THE ENVIRONMENT OF GENETICALLY MODIFIED HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 10 OF DIRECTIVE 2001/18/EC

(COMMISSION DECISION 2003/701/EC)

LOGO OF THE COMPANY OR RESEARCH INSTITUTE (OPTIONAL)

The report format shall be completed by the notifier. The notifier shall fill in the report format according to the proposed form (tick boxes and/or, as far as possible, specific keywords to use in text fields). The notifier shall illustrate as much as possible the reported data by means of diagrams, figures and tables. Statistical data could also be provided where relevant.

In the case of multi-sites, multi-events and/or multi-annual release(s), the notifier shall provide a general overview of the measures taken and effects observed for the full duration of the consent.

The space provided after each item is not indicative of the depth of the information required for the purposes of this report.

1. General information

1.1. European notification number: **B/CZ/14/01**

1.2. Member State of notification:

prof. Mgr. Jaroslav Miller, M. A., Ph.D.
rector of Palacky University in Olomouc

Professional advisor: Ing. Ludmila Ohnoutková, Ph.D.

1.3. Date of consent and consent number

Decision 21775/ENV/15 from March 23rd 2015

2. Report status

2.1. Please indicate whether, according to Article 3 of the present Decision, the current report is:

— the final report

— ~~a post-release monitoring report~~

— ☒ final ☐ intermediary

3. Characteristics of the release

3.1. Scientific name of the recipient organism: *Hordeum vulgare L.*

3.2. Transformation event(s) (acronym(s)) or vectors ([1]) used (if transformation event identity not available):

CKX (genetically modified spring barley with altered level of cytokinin dehydrogenase enzyme), four types of modifications: pEXP:CKX1, pEXP: CKX2, bGLU:CKX1, CKX1:RNAi.

3.3. Unique identifier, if available: CKX

3.4. Please provide the following information as well as the field(s) layout.

Geographical location(s) (administrative region and, where appropriate, grid reference)	Size of the release site(s) ([1]) (m^2)	Identity ([2]) and approximate number of GM higher plants per event actually released (number of seeds/plants per m^2)	Duration of the release(s) (from ... (day/month/year) until...(d/m/y))
Region: Olomouc **Municipality:** Olomouc – Holice **Cadastral territory:** Holice u Olomouce **Plot number:** 641227 **Plot identification :** 1721	2015: Without border sowing: 72 sq m With border sowing: 663 sq m	21 600 plants Sowing density: 200 seeds per sq m	01/04/2015-10/08/2017
	2016: Without border sowing: 276 sq m With border sowing: 1190 sq m	27 600 plants Sowing density: 100 seeds per sq m	
	2017: Without border sowing: 219.6 sq m With border sowing: 1230 sq m	43 800 plants Sowing density: 200 seeds per sq m	

4. Any kind of product that the notifier intends to notify at a later stage

4.1. Does the notifier intend to notify the released transformation event(s) as product(s) for placing on the market under Community legislation(s) at a later stage?

– ☐ Yes ☐ No ☒ Unknown to date

If yes, indicate the country(ies) of notification:.....

If yes, specify for which use(s):

— Import

— Cultivation (e.g. seed/planting material production)

— Food

— Feed

— Pharmaceutical use (or processing for pharmaceutical use)

— Processing for

  — Food use

  — Feed use

  — Industrial use

— Others (specify):.....

5. Type(s) of deliberate release(s)

Please select the main type(s) (in boxes) as well as subtype(s) of the release(s). In the case of multi-sites, multi-events and/or multi-annual release(s), please provide a general overview of the type(s) of deliberate release(s) which has/have been carried out for the full duration of the consent. Please tick the appropriate type(s):

5.1. Deliberate release(s) for research purposes ☒

The purpose of the project is to test the higher tolerance of bGLU:CKX1, pEXP:CKX1 and pEXP:CKX2 against drought and to test the overall yield of CKX1:RNAi and bGLU:CKX1 under optimal or water stress field conditions.

5.2. Deliberate release(t) for development purposes ☐

— Event screening

— Proof of concept ([2])

— Agronomic performances (e.g. efficiency/selectivity of plant protection product, yield capacity, germination capacity, crop establishment, plant vigour. plant height, susceptibility to climatic factors/diseases, etc.) (specify)

— Altered agronomic properties (e.g. disease/pest/drought/frost-resistance, etc.) (specify)

— Altered qualitative properties (prolonged shelf-life, enhanced nutritional value, modified composition, etc.) (specify)

— Stability of the expression

— Multiplication of lines

— Hybrid vigour study

— Molecular farming ([3])

— Phyto-remediation

— Others: .....(describe).....

5.3. Official testing ☐

— Variety registration on a national variety catalogue

— DUS (**D**istinctness, **U**niformity and **S**tability)

— VCU (**V**alue of **C**ultivation and **U**se)

— Others: (specify).....

5.4. Herbicide authorisation ☐

5.5. Deliberate release(s) for demonstration purposes ☐

5.6. Seeds multiplication ☐

5.7. Deliberate release(s) for biosafety/risk assessment research ☐

— Vertical gene transfer studies

— Out-crossing with conventional crops

— Out-crossing with wild relatives

— Horizontal gene transfer studies (gene transfer to micro-organisms)

— Management of volunteers

— Potential changes in persistence or dispersal

— Potential invasiveness

— Potential effects on target organisms

— Potential effects on non-target organisms

— Observation of resistant relatives

— Observations of resistant insects

— Others: (describe).....

5.8. Other(s) types) of deliberate release(s): ☐

(describe) .....

6. Method(s), result(s) of the release, management and monitoring measure(s) in respect of any risk to human health or the environment

6.1. Risk management measure(s)

Please report the risk-management measures, which have been used to avoid or minimise the spread of the GMO(s) outside the site(s) of release, and in particular those measures

— which were not originally notified in the application,

Any risk was not observed.

— which were applied in addition to the conditions in the consent,

Any risk was not observed.

— which the consent required only under certain conditions (e.g. dry periods, flooding),

Any risk was not observed.

— for which the consent allowed the notifier a choice among different measures.

Any risk was not observed.

Tick the examples where appropriate:

6.1.1. Before the sowing/planting:

— Clear labelling of the GM seeds/planting material lots (distinct from other seeds/tubers/etc.)

Seeds were labelled: "Genetically modified spring barley CKX, Do not use for food or feed", Seeds were placed into labelled sealed paper bags marked with a barcode the way that they could not be confused. The bags were placed into two special lockable plastic containers in the GMO laboratory, building H, the first floor above ground.

— Segregation during the processing and transport of the seed/planting material (describe the method involved; provide example(s) of containment to prevent spillage during the processing and transport)

Seed were transported in sealed, clearly marked paper bags placed in a double container. For the purpose of transport, two lockable, interlocked, plastic containers were used. The outer as well as the inner plastic container was able to prevent independently of each other spillage of the transported GM material.

— Destruction of superfluous seeds/planting material (describe the method involved)

The experiment was conceived the way that no seeds were left after the sowing.

— Temporal isolation (specify)

Was not implemented..

— Rotation (specify the previous crop(s))

— Other(s): (specify) 2015 – blue tansy, 2016 – maize, 2017 – blue tansy.

6.1.2. During the sowing/planting activities:

— Method of sowing/planting

In all years, GM seeds were planted separately one by one in rows with Hege sowing machine, which does not allow any remains.

— Emptying and cleaning of the sowing/planting machinery on the field of release

The sowing machine Hege does not leave any remains; all prepared seeds were sown every time.

— Segregation during the sowing/planting (provide example(s) of containment to prevent spillage during the sowing/planting)

Specific amount of seeds of particular progeny of each line were every time loaded into the sowing machine. After the sowing, the sowing machine was properly emptied and cleaned the way that different lines were not mixed together.

— Other(s) (specify).....

6.1.3. During the period of release:

— Isolation distance(s) (x metres)

— from sexually compatible commercial plant species,

100 meters

— from sexually compatible wild relatives.

Spring barley is a self-pollinating plant and it does not cross with wild species occurring on the territory of the Czech Republic.

— Border row(s) (with the same crop or a different one, with a non-transgenic crop, x metres, etc.)

Border row of wild-type spring barley 3.4 m wide was sown around whole GM plot at distance more than 100 m from commercially cultivated barley. Distance between border row and GM plants was 3 meters.

— Cage/net/fence/signpost (specify)

Immediately after the sowing, fencing of the experimental plot was carried out. Warning signs have been placed with the wording "ATTENTION - GMO - DO NOT ENTER - DO NOT CONSUME - CHEMICAL TREATMENT. Not specified for consumption, feeding! Prohibition of handling to unauthorized persons! "

— Pollen trap (specify)

Pollen traps were not used.

— Removal of GM inflorescences before flowering (indicate the frequency of the removal)

GM inflorescences were not removed, barley is self-pollinating plant.

— Removal of bolters/relatives/hybrid partners (indicate the frequency of the removal, x metres around the GM field, etc.)

Bolters of relative plants did not emerge.

— Other(s): (specify): none

6.1.4. At the end of the release:

— Harvest/destruction methods (of crop or parts of it)/other means (e.g. sampling and analysis of sugar beet pulp) (describe) Harvest /destruction before the ripeness of the seeds

The harvest was carried out by a small-scale combine harvester. The harvested seeds were stored in marked textile bags ordered by individual lines. Seeds were transported in a double container (two lockable, interlocked, plastic boxes). Plastic containers containing seeds of genetically modified spring barley have been labelled "Contains genetically modified material (spring barley CKX). Not specified for consumption, feeding! Prohibition of handling to unauthorized persons! "Samples from selected GM plants were placed into plastic tubes, coded, frozen and inserted into plastic containers. The collected samples were used for molecular analysis. Transport of samples of plants and harvested seeds was ensured by a competent and trained employee of the Faculty of Science, Palacký University in Olomouc.

Border barley row was disposed of at full maturity stage on the same day as GM plant harvest. All plant material was crushed on a mulcher on the same day. Immediately

after the harvest, N fertilizer ammonium nitre (LAV) fertilizer was applied at a rate of 30 kg per ha to support decomposing processes and all remaining plant material was introduced into the soil by a medium plough.

Harvesting was not done before the seeds maturation.

— Effective removal of plant parts

Parts of the plants have not been removed.

— Segregated storage and transport of crop/waste

The waste was not transported out of the field.

— Clean up of machinery on the release site

Harvesting was carried out by a small-scale combine harvester. The used small-scale combine harvester is specially designed for experimental purposes and meets all the necessary parameters. The harvested grain was stored in designated textile bags separately by each line.

— Destination of the waste, treatment of waste/surplus yield/plant residues

At the end of the experiment, all remaining plant mass, including border wild-type spring barley, was destroyed by crushing; nitrogen fertilizer ammonium nitre containing calcium (LAV) at 30 kg per ha and the decomposed plant residues were ploughed into the soil.

— Other(s): Not specified.

6.1.5. Post-harvest measures

Please indicate which measures were taken on the release site after the harvest:

Frequency of visits: Twice a week.

— Subsequent crop: 2015 – blue tansy, 2016 – maize, 2017 – blue tansy

— Crop rotation: Blue tansy – barley, maize – barley, blue tansy - barley

— Fallow/no crop: Not applied.

— Superficial soil work/no deep ploughing: Mulching of plant remains, deep ploughing

— False-sowing beds: Not applied.

— Control of volunteers: Once a month.

— Appropriate chemical treatment(s): Plants were treated by herbicide Mustang Forte and fungicide Acanto and Capalo.

— Appropriate soil treatment(s): Rotavatoring, dragging, ploughing, fertilizing (fertilizer Amops, KCl).

— Others: None.

Others) measure(s): (describe):

None.

6.1.6. Emergency plan(s)

Indicate:

(a) if the release proceeded as planned:

— Yes

— ~~No (describe for which reason, e.g. vandalism, climatic conditions, etc.)~~

(b) if measures according to the emergency plans) (Article 6(2)(a)(vi) and Annex III.B of Directive 2001/18/EC) had to be taken:

— No

— ~~Yes (describe):~~

6.2. Post-release monitoring measures

Due to the fact that the current report format can be used for the final and post-release monitoring report(s), the notifier is asked to clearly make the difference between both types of report through this section 2 of Chapter 6.

Please indicate whether

— **the post-release monitoring plan will start** (in the case of a final report, after the last harvest of the GM higher plants),

— ~~**the post-release monitoring plan is ongoing** (in the case of an intermediary post-release monitoring report),~~

— ~~**the post-release monitoring plan has been completed** (in the case of the final post-release monitoring report),~~

— ~~**no post-release monitoring plan has to be fulfilled**.~~

The results of this monitoring are meant to confirm or invalidate earlier assumptions in the risk assessment.

According to the aforementioned cases, please indicate which monitoring measure(s) will be/are/were taken and where (on the release site/near the site (e.g. on fields edges)). Please be aware that all post-release monitoring measures taken during the whole post-release period shall be indicated here.

Specify:

— Monitoring measures within site

Duration: 4 years.

Frequency of visits (average): Once a month.

— Observation of resistant relatives: Not observed.

— Observation of resistant insects — Control of volunteers (specify intervals and duration): Not performed.

— Monitoring of gene flow (specify): Was not proved.

— Appropriate chemical treatment(s) and/or soil treatment(s): Specified above.

— Others (specify): None.

— Monitoring measures of adjacent areas

Duration: One year after finishing the experiment

Frequency of visits: once a month due to monitoring plan

Area monitored:

— Observation of resistant relatives: This was not aim of the field trial.

— Observation of resistant insects: This was not aim of the field trial

— Control of volunteers and/or monitoring of feral populations: Once a month. Volunteers were not observed during ripening.

— Monitoring of gene flow: Was not proved.

— Appropriate chemical treatment(s) and/or soil treatment(s):

During the vegetation period, plants were treated by herbicide (Mustang Forte) and fungicides (Acanto, Capalo), during vegetation also fertilizer was regularly applied (ammonium nitre containing calcium (30 kg per ha). Soil was treated by rotavatoring, dragging and ploughing. Once a year, putative volunteers were eliminated by herbicide Kaput Harvest (4 liters per ha)

— Others (specify) None.

6.3. Plan for observation(s)/method(s) involved

In this section the observation plan and the methods used to collect the effects, which have to be reported under the next section (section 6.4), need to be specified. Any amendments or modifications to the plan as proposed in the application and the SNIF ([4]) part B need to be specified in detail.

During the time between the notification and the final report submission, new scientific insights or methods may be developed which cause a change in the methods used. In particular these modifications need to be specified under this section.

The observation methods were performed throughout the trial according to the approved methodology and monitoring plan.

During the field trial, the following phenological observations were made every year:

Evaluate the properties of GM plants and compared them with the properties of wild type barley (at least once every two weeks during the vegetation season), see field diary.

Monitoring of the individual stages of development of GM plants compared to control variants (as needed during the preparation phase of trial and at least once every two weeks during the trial period).

Observation whether there is inadequate release of the seeds from the spike (during ripening at least once a week).

Monitoring of volunteers at least once a month during the year following the end of the experiment.

6.4. Observed effect(s)

Any effect on environment was not observed which could be caused by interactions with non-target organisms as well as no negative effects on human health or animals were recorded.

Any non-expected morphological aspects of GM and wild-type barley were not found out, except enlarged root system of transgenic lines pEXP:CKX1, pEXP:CKX2 and bGLU:CKX1 and more robust tillering of CKX1:RNAi line.

6.4.1 Explanatory note

All results of the deliberate release(s) in respect of any risk for human health or the environment shall be stated, without prejudice to whether the results indicate that any risk is increased, reduced or remains unchanged.

The main objectives of the information given in this section are:

— to confirm or invalidate any assumption regarding the occurrence and impact of potential effect(s) of the GMO(s) which was/were identified in the environmental risk assessment,

— to identify effect(s) of the GMO(s) which was/were not anticipated in the environmental risk assessment.

The observed effect(s)/interaction(s) of the GMO(s)

— with respect to any risk to human health,

— with respect to any risk to the environment

shall be reported under this section.

Particular attention shall be drawn to unexpected and unintended effect(s).

Indications as regards the effects that the notifier may have to report are provided hereunder. The effects have obviously to be considered in the light of the crop, the new trait, the receiving environment as well as the conclusions of the environmental risk assessment, which is carried out on a case-by-case basis.

In order to structure the information and to facilitate an efficient search within the given information, the notifier shall use, as far as possible, specific keywords to fill in the text fields under Chapter 6, especially sections 6.4.2. 6.4.3 and 6.4.4. A most updated list of those specific keywords is available on the Internet at http://gmoinfo.jrc.it.

6.4.2. Expected effect(s)

This section concerns 'Expected effects', that is to say, potential effects which were already identified in the environmental risk assessment of the notification and could therefore be anticipated.

Notifiers should supply data from the deliberate release(s) which validate the assumptions made in the environmental risk assessment.

6.4.3. Unexpected effects ([5])

'Unexpected effects' refer to effects on human health or the environment, which were not foreseen or identified in the environmental risk assessment of the notification. This part of the report should contain any information with regard to unexpected effects or observations relevant for the initial environmental risk assessment. In case of any observed unexpected effects or observations, this section should be as detailed as possible to allow a proper interpretation of the data.

6.4.4. Other information

Notifiers are encouraged to supply information, which is outside the scope of the notification but which might be relevant to the field trials in question. This may also include observations of beneficial effects.

7. Conclusion

In this chapter, the notifier should specify the conclusions drawn and the measures taken or to be taken on the basis of the results of the release with regard to further release(s) and, where appropriate, make reference to any kind of product the notifier intends to notify at a later stage.

Spring barley with the introduced CKX genes of interest, resulting in increased or decreased cytokinin dehydrogenase (CKX) activity in the roots or spikes, which leads to a decrease or increase in the level of the plant hormone cytokinin, resulting in greater proliferation of root meristems and enhanced forming of tillers, followed by the development of a more potent root system and more spikes, and an increase in the resistance to drought and better nutrient intake. Field experiments included four types of CKX modifications: pEXP:CKX1, pEXP:CKX2, bGLU:CKX1 and CKX1:RNAi.

Experiment confirmed that some of the modifications led to a higher yield, especially under conditions of applied water stress.

No specific measures resulting from field trials with CKX transgenic barley are necessary. Cultivation of this barley is safe and has no negative effect on human health, animal health and the environment.

The information provided in this report is not considered confidential in accordance with Article 25 of Directive 2001/18/EC. This does not prevent the competent authority from requiring additional information from the notifier, both confidential and non-confidential. In the case of confidential data, it should be provided in an Annex to the report format, with a non-confidential summary or general description of these data, which will be made available to the public.

DATE: February 28, 2018