FORMAT FOR THE PRESENTATION OF THE RESULT OF DELIBERATE RELEASE INTO THE ENVIRONMENT OF GENETICALLY MODIFIED HIGHER PLANTS IN ACCORDANCE WITH ANNEX XI OF ROYAL DECREE 178/2004

1 General information

1.1 **European notification number:** ***B/ES/13/16***

1.2 **Member State of notification:** ***Spain***

1.3 Date of consent and consent number: ***18/04/2013 Catalonia B/ES/13/16***

2 Report status

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

The final report

3 Characteristics of the release

3.1 **Scientific name of the recipient organism:** ***Zea mays***

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

The event is named L1.
For the transformation experiment we have used as selectablo marker the bar gene and 4 cDNAs that encode for the metabolic pathway fror the production of β-caroteno, ascorbat and folic acid.
To increase the ***β-caroteno*** *content we have introduced the el fitoen sinthasa cDNA (psy1) from corn (Zea mays) driven by the LMW glutenin promoter from wheat and the crtI gen from Pantoea ananatis (before Erwinia uredovora) (that encodes for caroten desaturase driven by the D-hordein promoter from barley.*
To increase the ***ascorbate*** *concentration we have introduced the cDNA that encodes for dehidroascorbat reductase (dhar) from rice driven by the D-hordein promoter from barley.*
To increase the levels of ***folic acid*** ***we have introduced the*** *folE gen from E. coli that encodes for GTP ciclohidrolase (GCH1), driven by the D-hordein promoter from barley.*

[1] In the case of small-scale field trials where several lines may be tested, the vectors used should be mentioned, which gives insight into the introduced traits and/or genetic elements. In the case of large®-scale trials, the number of events notified is limited to only one or a few events.

3.2

3.3 **Unique identifier, if available:** ***L1***

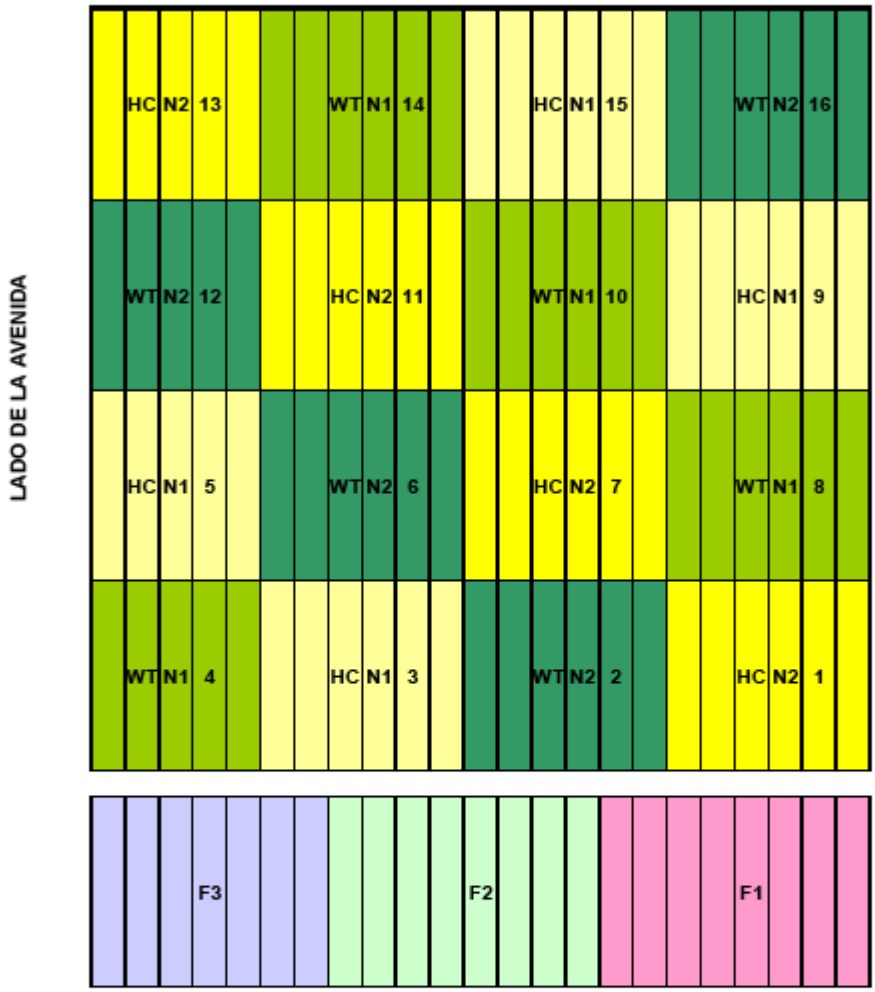
3.4 **Please provide the following information as well as the field(s) layout:**

Geographical location(s) (administrative region and, where appropriate, grid reference)	**Size of the release site(s) ([2]) (m2)**	**Identity ([3]) and approximate number of GM higher plants per event actually released (number of seeds/plants per m2)**	Duration of the release(s) (from … (day/month/year… until… (d/m/y)
Lleida municipality	***1400 m^2 1n total: 500 m^2 of L1 Surrounded by 75 m^2 of 3 control, 75 m2 of tester lines (named F1, F2 and F3) for crosses and surrounded by around 750 m^2 MON810***	***1600 transgenic plats***	***from* 06/05/2013 *until* 13/10/2013**

([2]) Specify the size of the GM area and, where appropriate, the size of the non-GM area (e.g. non-GM border)

([3]) Vectors used

ENSAYO MAÍZ CAROLIGHT LLEIDA 2013



LADO DE LA AVENIDA

LADO DEL CANAL

Legend: WTN1 = wild type low N; WTN2 = wild type high N; HCN1 = high carotenoid low N HCN2 = high carotenoid high N	**Latin square design with four reps** **Six-row plots** of 6,47 m length **Rows** spaced 0.65 m **No. plants per row** = 28 (approx; 4 plants per linear meter) **Total plants per plot** = 168 **Approx. plot size** = 3,9 m (width) x 6,47 m (large) = 25,2 m^2 **Approx. field dimensions** = 25,2 m^2 x 16 plots = 403,2 m^2

4 Any kind of product that the notifier intends to notify at 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 (by another juridical entity of the group) ☐ No *X* 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 pour.
    - 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** ***X***

5.2 **Deliberate release(s) for development purposes** ☐

- Event screening.
- Proof of concept [2].

[2] For example, testing the new trait under environmental conditions.

- 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 : (specify) .....

5.3 **Official testing** ☐

- Variety registration on a national variety catalogue

    - DUS (=Distinctness, Uniformity and Stability)
    - VCU (=Value of Cultivation and Use)

- Others: (specify): …….

5.4 **Herbicide authorization** ☐

5.5 **Deliberate release(s) for demonstration purposes** ☐

5.6 **Seeds multiplication** **X**

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

- Vertical gene transfer studies.

[3] « Molecular farming » means the production of substances (for instance, proteins, pharmaceuticals) by plants, which have been genetically modified for a particular trait. “Molecular farming” could be defined as well as the production of plant-synthesised pharmaceuticals, plant-made pharceuticals, plant-based proteins production, etc.

- 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.

- Observation of resistant insects.

- Others: (describe) .....

-

5.8 **Other(s) type(s) 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,

- Which were applied in addition to the conditions in the consent,

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

- For which the consent allowed the notifier a choice among different measures.

Tick the examples where appropriate:

6.1.1 Before the sowing/planting:

- Clear labelling of the GM seeds (distinct from other seeds/tubers/etc.) (describe).

The GMO's seeds were pack in 8 individual envelopes with 200 seeds each and label with a black marker indicating line HC (L1, Carolight). The rest of the corn lines used as control were also packed in 8 envelopes with 200 seeds each and label WT. Bags with parental seeds for crosses contained also 200 seeds each and were label parental F1, parental F2 and parental F3.

- 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).

All seeds were transported by hand, all of them in a plastic box fully sealed.

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

The few L1(Carolight) left over seeds were taken back to the laboratory of Applied Plant Biotechnology (Laboratory 1, Building AB, underground floor) and stored together with the other from the same lane. These are very important seeds that we cannot destroy for now.

- Temporal isolation (specify).

We carry out the sawing of the L1 seeds three weeks later of the one used as a border lane.

- Rotation (specify the previous crop).

- Other(s): (specify)

6.1.2 During the sowing/planting activities:

- Method of sowing/planting.

We used a sowing machine. We took 1 hour.

- Emptying and cleaning of the sowing machinery on the field of release.

Cleaning was done air pressure and we confirmed no presence of seeds at the end of the cleaning.

- Segregation during the sowing (provide example of containment to prevent spillage during the sowing/planting).

Each seed bag was open in the specific site just before planning, and after confirmation that the label coincided with the specific planning site in the plot, we proceed to the sowing and we confirmed that the sowed seeds were as indicated in the original plan.

- Other(s): (specify)

6.1.3 During the period of release:

- Isolation distance (x meters)

- From sexually compatible commercial plant species.
- From sexually compatible wild relatives.

There were not sexually compatible commercial plants of wild relative, only the corn used as a border raw.

- Border rows (with the same crop or a different one, with a non-transgenic crop, x meters, etc).

As a border raw we saw 750 m^2 of MON810 surrounding the L1 (Carolight) seeds.

- Cage/net/fence/signpost (specify).

- Pollen trap (specify).

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

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

- Other(s): (specify)…….

6.1.4 At the end of the release:

- Harvest/destruction methods (of crop or part of it) / other means (e.g.: sampling) (describe).

- Harvest / destruction before the ripeness of the seeds.

- Effective removal of plant parts.

- Segregated storage and transport of crop/waste (provide examples of containment to prevent spillage of collected seeds/crops/wastes).

We hand harvest the L1 material the 13/11/2013 and we stored the seeds in plastic boxes, which were previously label and sealed for transportation to the laboratory. The Catalan de Biosegurity Committee was informed of the harvest day with more then 5 days in advanced, which allowed to the commission to send a representative.

- Clean up of machinery on the release site.

- Destination of the waste, treatment of waste/ surplus yield/plant residues (describe).

Grains were separated from stocks using an electric disgraner that was cleaned carefully after finishing the process. Previously we had slightly dried the corn cops in an oven at 40 ºC.

The stocks were autoclaved in the laboratory and packet in plastic bugs hermetically sealed. These bags were further deposited in sealed containers that are collected by the residues services, following the protocol for discarding biological material.

The harvested seeds from L1 were stored in the cabinet in the laboratory of Applied Plant Biotechnology (Laboratory 1, Building AB, underground) chosen for this specific purpose.

- Post-harvest treatment and cultivation measures on the release site (describe the method for preparing and managing the release site at the end of the release, including cultivation practices).

Once the crop harvest finished, we inspected the field during three consecutive days and we collected manually all the grains we could find. Those grains were stored in a plastic bag and finally autoclaved. Those leftover autoclaved grains were placed in the biological material containers ready for the specialized disposal company to take them.
The harvest of the 750 m^2 from MON810 planted around the field trial was carried out in desember 11, 2013. All those grains were destroyed by burial in the ground on a 5 m^2 whole open for this purpose. The Catalan Biosegurity Commission was informed of the harvest of this conventional crop more the 5 days in advance, which allowed the visit of a representative form the commission
The dry leaf and part of the plant left and the surrounding conventional crop were buried with mechanical tractor application.

- Other(s): (describe):

Yes, we have a field notebook were all procedures are described in detail, since planning until destruction of the material.

6.1.5 Post-harvest measures:

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

Frequency of visits (average)
Within the post release monitoring we are going to visit the site of the field trial, and we are already doing it, once a moth during the whole next planning season.
- Subsequent crop (specify).

We will not grow corn, probably a collection of wheat will be planted

- Crop rotation (specify).

- Fallow/no crop (specify).

- Superficial soil work / no deep ploughing.

Work of disc plough and chisel

- False-sowing beds.

- Control of volunteers (specify intervals and duration).

- Appropriate chemical treatment(s) (specify).

- Appropriate soil treatment(s) (specify).

- Other(s) (specify)

6.1.6 Other(s) measure(s): (describe)

6.1.7 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 plan(s) (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 spot release monitoring is in place and we will keep it for one year. We will include the explanations within the report presented for next year release.

- **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: *1 year*

  Frequency of visits (average): *once a month*

- Observation of resistant relatives.
- Observation of resistant insects.
- Control of volunteers (specify intervals and duration). If volunteers are observed they will be pull out manually and sure before pollination.

- Monitoring of gene flow (specify).
- Appropriate chemical treatment(s) and/or soil treatment(s).
- Others (specify).

- Monitoring measures of adjacent areas:

  Duration:

  Frequency of visits (average):

  Area monitored:

  - Observation of resistant relatives.
  - Observation of resistant insects.
  - Control of volunteers and/or monitoring of feral populations (specify intervals and duration).
  - Monitoring of gene flow (specify).
  - Appropriate chemical treatment(s) and/or soil treatment(s).
  - Others (specify).

6.3 **Plan for observation(s)/methods(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.

6.4 **Observed effect(s)**

The surrounding area of the GM field was 10 times bigger then the field per se, at the same time, the experiment was carried out within the city area with no growing fields, all this helped the preempt any interaction that could cause any risk for the environment. No risk for human health has been detected. The already expected cross pollination was buffered for all the plant that surrounded the field. All rest of seed and material was destroyed and eliminated. During the whole process we did not detect any unexpected problem.

[4] Summary notification information format (=SNIF)

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 and 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 effect(s) [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.

1. The field trial has been carried out as planned in the notification and we have carried out all the measures for risk control.
2. The Catalan Biosegurity commission has been always notified with anteriority to the flowering of the crops and always in advance to any action taken by the research group.
3. We have followed al the specifications required by the Catalan Biosegurity Commission and we have carried out all the inspections required during sowing, growing and harvesting.
4. We have achieved the main objective that was to amplify L1(Cariloght) seeds
5. We have established a post harvest monitoring plan for 1 year
6. We did not detect any adverse effect for the human health or environment.

DATE: *22/01/2014*

[5] Without prejudice to Article 8 OF Directive 2001/18/EC as regards handling of modifications or new information.