

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/13/20

1.2 Member State of notification: Spain

1.3 Date of consent and consent number: 11/04/13

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: *Triticum aestivum*

3.2 Transformation event(s) (acronym(s) or vectors¹ used (if transformation event identity not available):

It has been used the transformation event (line) E82. Among all the transformation events, it was the one which showed higher levels of reduction in both gliadin content and gluten content, as determined by R5 and G12 monoclonal antibodies. Both antibodies detect a higher than 95% decrease in the flour gluten content of E82 line. It is noteworthy that the gluten content in E82 line decreases further (18%) when it is processed into flour to make bread. In the case of the selected wheat line E82, studies with T cells that specifically recognize the peptide 33-mer showed a 100-fold reduction in the toxicity of the proteins from the flour of this line as compared to control positive. The gluten from the E82 line was incapable of stimulating T cells that recognize other epitopes present in γ -gliadin and ω -gliadins.

3.3 Unique identifier, if available: Wheat E82

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

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.

Geographical location(s) (administrative region and, where appropriate, grid reference)	Size of the release site(s) (²) (m ²)	Identity (³) 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)
Fuente Palmera (Córdoba)	2000 m ² (effective sown area: 1152 m ²)	130 seeds/m ² of E82 wheat	01/December/2013 13/June/2014

(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

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 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

5.2 Deliberate release(s) 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 farming³.
- Phyto-remediation.

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

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 pharmaceuticals, plant-based proteins production, etc.

- 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

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.
- Observation of resistant insects.
- Others: (describe)
-

5.8 Other(s) type(s) of deliberate release(s):

X

The release has been aimed at producing 500 kilograms of grain to conduct a clinical trial with flour at the Reina Sofía Hospital in Córdoba. The tested wheat line has very low reactivity in relation to celiac disease as reported by T cells previously made (PNAS, 2010 107: 17023-17028), and the results of monoclonal antibodies currently available.

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)

All the measures included in the application have been carried out. There have been no special circumstances that indicate the need for further action.

6.1.1 Before the sowing/planting:

A quantity of 4800 g of wheat seed E82, previously produced in greenhouses confinement at the Institute of Sustainable Agriculture, was used. The seed was transported to the plot in double bag in a vehicle of the institution. At no time during transport seed losses occurred.

In the previous season, the trial plot was cultivated with sunflower. Preparatory work normally carried out in the farm (disc harrow, chisel, and vibroculcator) were made prior to planting. Finally, 48 rows of 48 m long spaced 50 cm apart were marked.

6.1.2 During the sowing/planting activities:

The double bag containing the seed was opened on the trial site. The seed available was used in its entirety. Sowing was carried out with a manual sowing machine on 1 December 2013. The seeding rate was 65 seeds per meter.

After the completion of the sowing, all components of the sowing machine were cleaned with an air compressor on the plot. Operators responsible for the sowing were equipped with disposable bodysuits and overshoes which, together with the bags which had contained the seeds, were stored in sealed autoclave double bags. These bags containing waste were sterilized before being eliminated.

6.1.3 During the period of release:

A perimeter fence 1.5 m high was installed surrounding the entire trial to prevent the entry of small animals. Between the fence and adjacent crop, rapeseed, a distance of 2 m was left. The nearest compatible cereal was at a distance greater than 200 m.

All test area was covered with anti bird mesh prior to anthesis, and throughout the cultivation period the integrity of the barriers was monitored.

6.1.4 At the end of the release:

The harvest took place on June 13, 2014, counting at all times with the presence of Domingo Ota, representing the Ministry of Agriculture (MAGRAMA).

The grain was harvested with a Wintersteiger experimental plots harvesting machine. Once finished, a visual inspection was performed to remove from the area any remaining ear. The grain of each pass of the harvesting machine was double bagged. Each of these bags was numbered, stitched to avoid grain losses, weighed and marked as containing GMOs by the afore mentioned inspector. A total of 395 kg of grain was obtained.

The palletized bags were stored until grinding in a dependence of the farm, in a clean room, padlocked, with no access to unauthorized personnel. Appropriate measures were established to prevent the entry of rodents. A sign noting the existence of GMOs and the prohibition of passage was fixed at the entry door.

The whole harvested grain was milled in an experimental mill, in presence of the MAGRAMA inspector at all times, ensuring that no seed could be unintentionally released. All the obtained flour (280 kg of white flour) has been stored.

Subsequently all debris from the grinding were incinerated in the presence of inspector MAGRAMA.

6.1.5 Post-harvest measures:

The test plot was ploughed immediately after harvesting. Weekly visual inspections were carried out to monitor the seedling emergence of genetically transformed wheat plants, which occur after the first rains in autumn. All germinated plants were eliminated with a total herbicide (glyphosate) and, subsequently, the preparatory work was performed, both in the trial plot and the adjacent area, prior to sowing rapeseed in late October. During rapeseed cultivation, an herbicide to control narrow-leaved plants was applied. No wheat plant was observed in any of the inspections conducted.

A disc harrowing has been carried out after the harvesting of the rapeseed. Visual inspections to detect wheat plants will continue, particularly after rains and glyphosate will be reapplied, when necessary, to eliminate volunteer wheat that could germinate. In the next cropping season, we plan to plant corn on the plot, making easier the control of adventitious flora because the soil is left bare all winter.

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

Not applicable.

6.1.7 Emergency plan(s).

Not applicable.

6.2 Post-release monitoring measures

The post-release monitoring plan has been completed

6.3 Plan for observation(s)/methods(s) involved

Not applicable.

6.4 Observed effect(s)

Not applicable reporting of results relating to risk assessment.

7 Conclusion

The yield has been less than the 500 kg of grain originally planned to conduct the clinical trial. However, with this grain yield 280 kg of flour were obtained , which according to initial estimates are sufficient to conduct the clinical trial.

DATE: Córdoba, June 15, 2015