Deliberate release B/BE/11/V4

Final report

1. General information

1.1. European notification number
B/BE/11/V4.

1.2. Member state of notification
Belgium

1.3. Date of consent and consent number
10 February 2012, B/BE/11/V4

2. Report status
This is a final report.

3. Characteristics of the release

3.1. Scientific name of recipient organism
Zea mays (common name: maize)
The maize plants were modified to overexpress an enzyme involved in the biosynthesis of certain gibberellin plant hormones.

3.2. Transformation event(s) (acronym(s)) or vectors used

Line n°	Parent line	Construct	Gene of interest	Marker gene
GA20OX1(R)	B104	pBbm42GW7-pUBIL-GA20ox	*GA20oxidase1*	*bar*

3.3. Unique identifier
The line has not been given a unique identifier number.

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

Geographical location(s)	Size of the release site(s) (m^2)	Identity and amount of GM plants per event released	Duration of the release
2012			
Municipality of Wetteren	Including non-GM borders and reference lines approximately 390 m^2 (of which 48m^2 GM plants)	Identity: See 3.2. Amount: approximately 10 plants/m^2	25 May to 17 October 2012
2013			
Municipality of Wetteren	Including non-GM borders and reference lines approximately 900 m^2 (of which 162m^2 GM plants)	Identity: See 3.2 Amount: approximately 10 plants/m^2	29 April to 11 October 2013

The field trial plot design is given in annex.

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?
No.

5. Type of deliberate release
This was a deliberate release for basic research purposes. The goal was to see whether or not certain phenotypical changes that had been observed in a greenhouse would also appear under agricultural conditions.

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 measures

6.1.1. Before planting
Measures such as appropriate packing and clear labeling were taken to preserve the correct identity of the GM maize and to prevent the spread or admixture of GM maize.

6.1.2. During planting activities
Same measures as before planting. GM seeds were manually sown by personnel that received compliance training. Any left-over seeds were carefully packed and labeled and returned to the research facility.

6.1.3. During the period of release
The field trial location is surrounded by a fence to prevent any unwanted trespassing.
The seeds were treated with a substance to prevent birds eating the seeds and the trial site was covered with bird netting until the plantlets had properly emerged.
There was a border of 4 rows of non-GM maize plants surrounding the GM maize plots, which created an isolation distance between the GM maize plots and surrounding maize of 3 meters. Male flowers of the GM plants and the non-GM comparator were carefully removed manually before they could start shedding pollen.

6.1.4. At the end of the release
Maize plants were carefully harvested manually and all cobs (even the smallest ones) were carefully removed. Cobs were doubly packed and correctly labeled and transported to the research facility for measurements and were destroyed afterwards. Roots were left on site for decomposition. Stalks and leaves were chopped on the plots and left for decomposition. Some samples were packed and labeled and transported to the research facility for measurements and destroyed afterwards.

6.1.5. Post-harvest measures
Field trial plots were left bare for one year to be able to spot any volunteer maize plants. Monitoring for volunteers took place from May until the end of August twice per month.

6.1.6. Other measures
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6.1.7. Emergency plan

The field trial took place according to plan. There were no disruptions of the trial. However, at the end of May 2013 there were traces of a break-in into the field trial premises. The wired fence had been cut at one specific location and there were traces of trespassing towards the maize field trial plot. There were no visual signs of disturbance of the field trial. Samples were taken to determine whether any unwanted substances such as herbicides had been applied to the trial site, but all samples were negative. The wired fence has been repaired immediately.

6.2. Post-release monitoring measures

The post-release monitoring measures will continue in 2014. In 2013 post-release monitoring took place on the 2012 trial plot location, resulting in no volunteer maize plants being detected.

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

During the first part of the season the field trial plot was visited weekly to check on the general situation of the trial and the health of the maize plants. In 2012 from 1st of July onwards until the moment that the first male flower appeared (23 August 2012) the trial was visited three times a week, and from the moment the first male flower appeared the trial was visited daily to check on and remove any male flowers. In 2013 the same regime was installed with the difference that the weekly visits took place until the beginning of August, the moment that the last leaf was formed. The first male flower was observed on 7 August 2013. From that moment onwards the trial was visited daily to check for male flowers and remove them. In the season after the release the trial plot is being monitored from May until the end of August on a bi-weekly basis to check on any maize volunteers appearing. This post-harvest monitoring will continue in 2014 for the 2013 trial plot.

6.4. Observed effects

There were no observations providing any indications of risks for human health or the environment.

6.4.1. Expected effects

The genetically modified maize plants were significantly higher, had longer leaves, and showed a higher cob implantation , , when compared to its non-GM counterpart. Although all these parameters were significant, the cob implantation was increased with less percentage as the final plant height, which can be considered as a positive trait as higher implantation of the cobs could result in increased tendency to lodging. Taken together, the phenotypes observed in the greenhouse were also observed in the field, and the extent of which was correlated to the environmental conditions (see annex for summary table of the phenotypes).

6.4.2. Unexpected effects

During the growth season 2012, there were signs of a less developed root system of the GM plants, which could cause lodging, although the genetically modified plants were not significantly more prone to lodging as compared to their non-GM siblings. . This observation was not seen in the growth season 2013, so no conclusions could be drawn here.

During the two growth seasons we daily monitored the flowering and noticed that the genetically modified plants flowered significantly later than the non-modified siblings, an observation that could not be made in the greenhouse.

6.4.3. Other information

From these field trials we learned that the inbred host line B104, which is used routinely in our Department for transformation, is not well adapted to the Belgian climate as the growing season is too short to obtain filled cobs. However, from the developing ears we were able to extract information on the potential seed set and ear length. The genetically modified plants had similar ear length and spikelets formed (the number of rows and the number of spikelets per row), showing that the transgene had no significant effect on seed yield parameters (see annex for summary table of the results). These are parameters which are more reproducible to measure under field conditions as compared to the greenhouse.

Finally, the daily monitoring of the appearance of the male flowers (tassels) learned us that there is a window of at least 3 to 4 days for a male flower to develop into a pollen-shedding flower, showing that daily detasseling is not necessary and could be weakened to once every two or three days in the future. In addition, it is also not necessary to daily monitor for flower formation until the harvest in late September or October as the very late-formed tassels never reach the pollen shedding stage and also because there are no fertile female flowers in neighboring fields present anymore at that time.

7. Conclusion

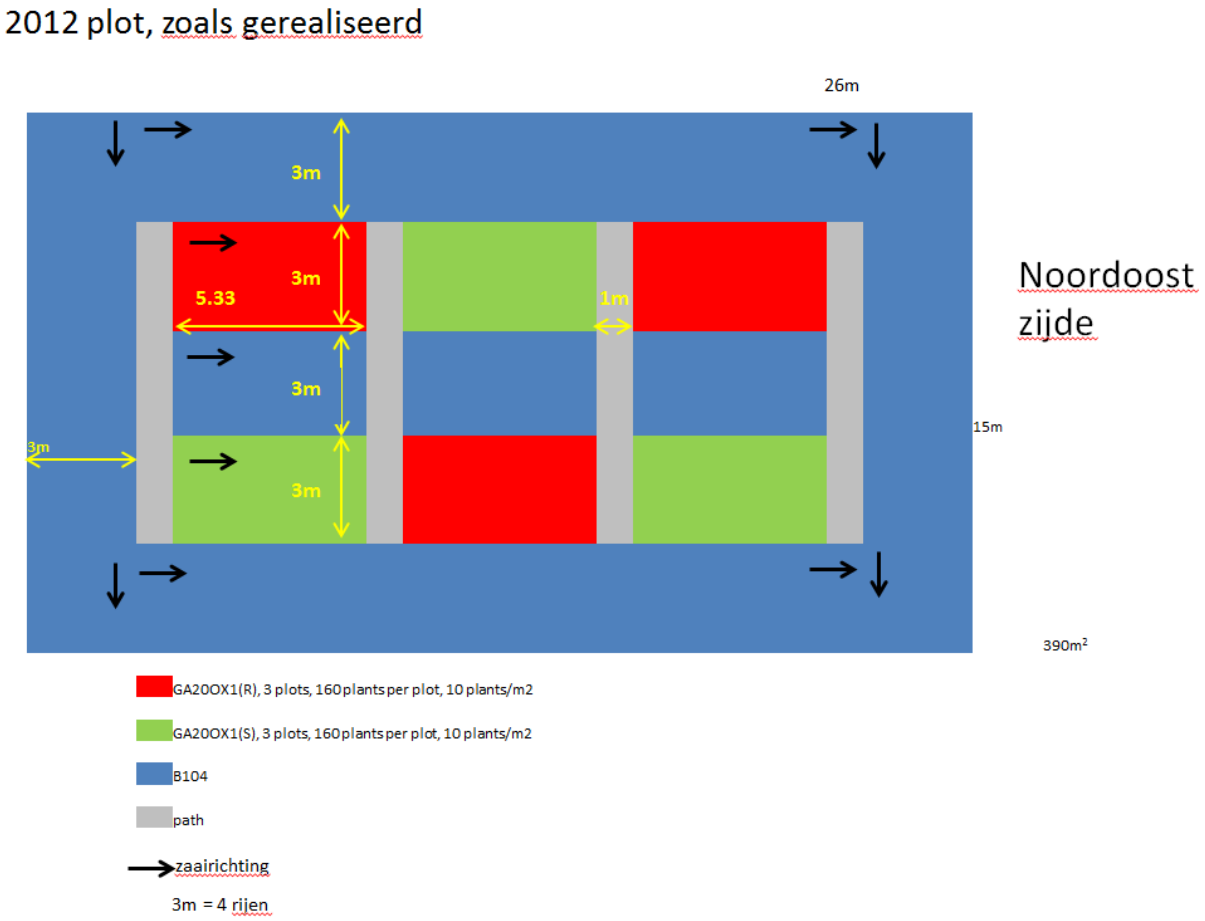
With the field trial it has been possible to confirm the greenhouse observed phenotypes of this maize line under normal agricultural conditions. Due to unfavorable climatic conditions it was not possible to determine whether or not the observed phenotype would allow for use in a higher planting density.

There are no indications whatsoever of any impacts on human health or the environment resulting from the field trial.

Date: 26 March 2014

Annex:

Field trial plot 2012:



GA20OX1(R) is the GM line.
GA20OX1(S) is the non-GM segregant (the near-isogenic comparator)

Trial plot design 2013

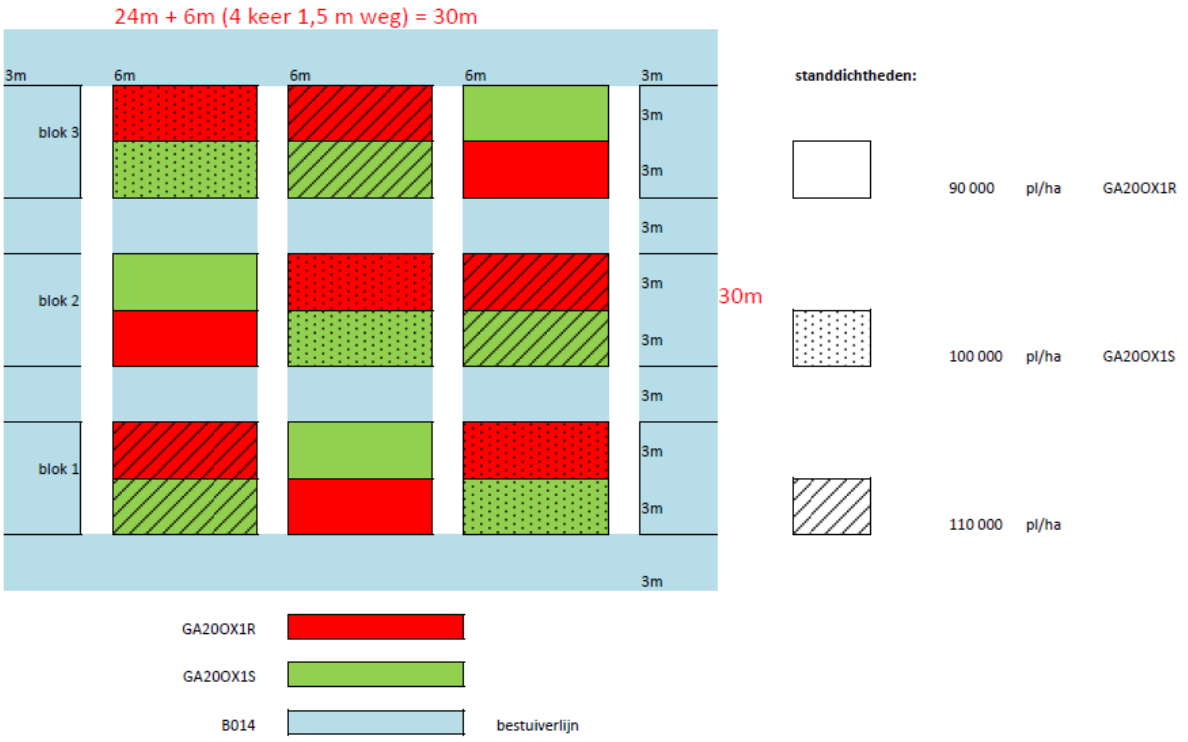


Table 1. Summary of the most relevant observed field trial parameters over the two growth seasons. The parameters were quantified and represented as the percentage relative to the non-modified siblings. The * indicates if the observed parameter was significant with a p-value < 0.01.

		growth season	
		2012	2013
parameters	final plant height	138.7%*	115.7%*
	cob implantation	120.3%*	106.4%*
	final length leaf 4	159.9%*	155.5%*
	number of spikelet rows	105.6%	100.4%
	number of spikelets per row	101.3%	103.6%
	ear length	101.1%	102.5%