

Cultivation of genetically modified sugar beet H7-1

COGEM advice CGM/091026-01

The present application by KWS Saat AG and Monsanto Europe S.A. (file EFSA/GMO/DE/2008/63) concerns cultivation of the genetically modified sugar beet H7-1.

Sugar beet H7-1 expresses the cp4 epsps gene. As a result this sugar beet is tolerant to glyphosate containing herbicides.

Sugar beet volunteers are almost never formed outside the field. However, in an agricultural environment volunteers may be formed from the seed that is produced by bolters, but also from plant crowns or portions of roots that are left on the field after harvest. In the soil, sugar beet seed can survive for a long period and may form a source of volunteer weeds. There are no indications that sugar beet can form feral populations outside an agricultural environment and there is no reason to assume that H7-1 sugar beet has an increased fitness or an increased potential to establish feral populations. Sugar beet may fertilize other beet species. Therefore, the glyphosate tolerance trait can be introduced into other cultivated beet species, weed beets and into wild sea beet. Glyphosate tolerance will only provide a selective advantage in an agricultural environment when glyphosate is used and will not provide a selective advantage under other conditions. Other herbicides will remain available that can be used to control weedy glyphosate tolerant beets. These herbicides cannot be used in a sugar beet crop, because sugar beets are also sensitive to these herbicides. COGEM points out that this situation is comparable to the current situation, where conventional weed beets cannot be controlled when sugar beet is cultivated.

COGEM is of the opinion that the molecular characterization of H7-1 sugar beet contains shortcomings. A small unexplained fragment appears to be present in the Southern blot that was carried out to detect backbone fragments. An explanation for the presence of this fragment should be provided. In addition, the applicant states that the basepairs flanking the cp4 epsps expression cassette correspond to sugar beet genomic DNA. This is not substantiated with data. The applicant should provide analyses that show the identity of the flanking regions of the cp4 epsps expression cassette. Furthermore, the bioinformatic analysis that was carried out on the junctions between the cp4 epsps expression cassette and its flanking regions was performed in 2003. The applicant states that the sequence of the cp4 epsps expression cassette may not be used for analysis in non-secured databases. COGEM is of the opinion that it is unacceptable that an old BLAST analysis is provided and that the provided sequences may not be analysed.

Furthermore, the General Surveillance plan should be improved on several points. The farm questionnaire should be complemented with additional questions. In addition, in order to allow detection of delayed and/or indirect effects, General Surveillance should also be carried out after cultivation of sugar beet H7-1. Therefore, if sugar beet H7-1 is cultivated until the end of the authorization period, General Surveillance should not be terminated at the end of the authorization period but should be continued for a prolonged period.

In conclusion, COGEM is of the opinion that the file concerning cultivation of sugar beet H7-1 contains inadequacies. The applicant should provide information on the remaining issues

before a decision on the authorization for cultivation of H7-1 sugar beet is taken by the competent authorities.

Introduction

The scope of the present notification (EFSA/GMO/DE/2008/63) by KWS Saat AG and Monsanto Europe S.A. concerns the cultivation of H7-1 sugar beet. Sugar beet H7-1 constitutively expresses the *cp4 epsps* gene, resulting in tolerance to glyphosate containing herbicides.

In the United States of America and Canada, H7-1 sugar beet was authorized for cultivation in 2005.¹ H7-1 sugar beet has been authorized for food and feed purposes in the European Union since October 2007.² The current application concerns cultivation of sugar beet H7-1. COGEM has not issued a previous advice on sugar beet H7-1.

Aspects of the crop

Sugar beet (*Beta vulgaris* subsp. *vulgaris*) is a cultivated form of *Beta vulgaris*. Sugar beet is used for the production of sugar and is cultivated world-wide.³ In the tropics, it is cultivated at higher altitudes because high temperatures adversely affect growth.⁴

In the first year of growth, sugar beet forms a rosette of leaves and a large taproot. Sugar beet is a biennial plant that needs a cold period (vernalization) to induce flowering. Generally, an inflorescence bearing stem (bolter) is not produced until the second year of growth.⁴ Cultivated sugar beets are harvested at the end of the first growing season and, therefore, usually do not flower. Nevertheless, bolters may occur in a sugar beet field. These bolters result from sugar beet plants that have been vernalized during the growth season or from contaminations in the seed that was used to sow.⁵ Seed used for sowing may contain traces of seed resulting from crosses between the cultivated sugar beet and the wild sea beet (*Beta vulgaris* ssp. *maritima*) in some seed production areas.⁶

If bolters are allowed to set seed large numbers of seeds are produced. These seeds can survive for a long period in the soil and may remain viable for eight³ to ten years.⁶ Under favorable conditions the seeds may germinate and form so-called weed beets. In the Netherlands, some of the fields that have been used for sugar beet cultivation contain large (stable) populations of weed beets.⁶ Weed beets can be controlled by several different herbicides. These herbicides can, however, not be used when sugar beets are cultivated because weed beets and sugar beets are sensitive to the same herbicides.

Besides volunteers that originate from seed, volunteers may also arise from plant crowns or portions of roots that are left on the field after harvesting. These plant parts can sprout and survive mild winters. However, fields are often ploughed before winter which prohibits the formation of volunteers from plant crowns and root portions.⁶ Occasionally, seed and/or portions of roots land outside the field on roadsides or on the edge of a field. Theoretically, volunteers could also arise outside the field. In practice, volunteers are almost never formed outside the field because beet parts usually decompose during winter and seedlings are very vulnerable to competition from the existing vegetation.^{5,6} There are no indications that sugar beet can form feral populations outside an agricultural environment.^{3,5}

Sugar beet is strongly self-incompatible and fertilization of the sugar beet thus occurs through cross-pollination.³ Sugar beet pollen is spread by wind and may travel large distances (8 to 9 kilometers).^{3,6} Occasionally the pollen is spread by insects e.g. thrips, honeybees, thrips.³

In the Netherlands, several close relatives of sugar beet, e.g. fodder beet (*Beta vulgaris*), chard (*Beta vulgaris* var. *cicla*), spinach beet (*Beta vulgaris* var. *cicla*) and beetroot (*Beta vulgaris* subsp. *vulgaris* var. *vulgaris*), are cultivated. In addition, the wild relative sea beet (*Beta vulgaris* subsp. *maritima*) is rarely found in the coastal regions of the Netherlands.⁷ Sugar beet can fertilize and produce fertile offspring with all the above-mentioned sub-species and varieties.^{3,5}

Molecular characterization

Agrobacterium tumefaciens mediated transformation was used to introduce the *cp4 epsps* expression cassette in sugar beet H7-1. As a result, the following sequences were introduced:

- 35S FMV promoter,
35S promoter from a modified *Figwort mosaic virus* (FMV)
- *ctp2* chloroplast targeting sequence,
the N-terminal chloroplast transit peptide sequence from the *Arabidopsis thaliana epsps* gene
- modified *cp4 epsps* gene,
the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) coding sequence from *Agrobacterium* sp. strain CP4, which was modified to enhance expression in plants
- E9 3' polyadenylation signal,
the 3' end from the *Pisum sativum rbcS* E9 gene
- left border,
enables the transfer of T-DNA and was derived from the pTi15955 plasmid of *A. tumefaciens*

Molecular analysis

Southern blot analysis with several probes spanning the vector backbone was used to determine whether (part of) the vector backbone is present in sugar beet H7-1. Based on this analysis the applicant states that sugar beet H7-1 does not contain any detectable backbone DNA from the transformation vector. However, backbone probe 3 appears to hybridize to a small fragment suggesting that a small backbone fragment might be present in H7-1 sugar beet. The applicant does not provide an explanation for the presence of this fragment. Therefore, it is uncertain whether backbone fragments are absent in sugar beet H7-1.

The applicant shows by Southern blot analysis that sugar beet H7-1 contains one copy of the *cp4 epsps* expression cassette. Southern blot and sequence analysis demonstrate that this single copy maintained its integrity, i.e. that the organization of the elements of the *cp4 epsps* expression cassette in H7-1 is identical to that in the transformation vector. According to the applicant the insert in H7-1 sugar beet is approximately 3.4 kb.

The sequence of the *cp4 epsps* expression cassette in H7-1 was compared to the transformation vector. This comparison identified differences for four nucleotides. Three of the differences are located in the non-coding region. The other difference is situated in the *cp4 epsps*

coding region, but does not change the amino acid sequence of the EPSPS protein. The comparison also showed that the left border end of the expression cassette in H7-1 sugar beet is truncated and that the right border end is not present.

The applicant sequenced part of the regions that flank the *cp4 epsps* expression cassette and states that these basepairs correspond to sugar beet genomic DNA. However, this conclusion is not substantiated with data. Therefore, it is not clear whether the sequences that flank the *cp4 epsps* expression cassette are sugar beet genomic DNA or whether other sequences have co-inserted with the expression cassette.

The applicant analysed the sequences spanning the junctions between the *cp4 epsps* expression cassette and its flanking regions. Sequences were translated *in silico* from stop codon to stop codon in all six reading frames and the putative polypeptides from each reading frame were analysed for homology to allergens, toxins or pharmacologically active proteins. No biologically relevant structural similarities were identified. However, these analyses were performed in 2003 and have not been updated. The number of sequences that are available in databases increases very rapidly. The homology of the sequences to these recently added sequences was not studied. In addition, the applicant states that the sequence of the *cp4 epsps* expression cassette may not be used for analysis in non-secured databases, such as publicly accessible databases or internet-supported databases. In practice, this means that the applicant prohibits the analysis of the sequences with recent databases.

Properties of the introduced gene conferring herbicide tolerance

H7-1 sugar beet expresses the *cp4 epsps* gene which encodes a modified 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme. The EPSPS enzyme is involved in the biosynthesis of aromatic amino acids.

In non-transgenic plants glyphosate inhibits EPSPS and, therefore, interferes with the biosynthesis of aromatic amino acids. Application of glyphosate thus leads to a lack of amino acids essential for growth and development of plants and ultimately causes plant death.⁸ H7-1 sugar beet produces a modified EPSPS protein, which is not inhibited by glyphosate, and is therefore tolerant to glyphosate containing herbicides.

Environmental risk assessment

Sugar beet volunteers may originate from the seed that is produced by bolters, but can also arise from plant crowns or portions of roots that are left on the field after harvest. In the soil, sugar beet seed can survive for a long period and form a source of volunteer weeds.³ Volunteers are almost never formed outside the field.⁶ There are no indications that sugar beet can form feral populations outside an agricultural environment.³

H7-1 sugar beet expresses the *cp4 epsps* gene, thus conferring tolerance to glyphosate containing herbicides. There are no indications that introduction of the glyphosate tolerance trait enhanced the fitness of sugar beet H7-1 in absence of glyphosate.

Several close relatives of sugar beet are cultivated in the Netherlands, such as fodder beet, chard, spinach beet and beetroot. In addition, a wild relative, sea beet, is rarely found in the coastal regions of the Netherlands.⁷ When bolters of sugar beet H7-1 are not removed in time, sugar beet H7-1 may fertilize and introduce the glyphosate tolerance trait into the other beet

species that occur in the Netherlands.³ In addition, if bolters of sugar beet H7-1 are allowed to set seed or pollinate weed beet, glyphosate tolerant weed beets may be formed.

Glyphosate tolerance only gives a selective advantage when glyphosate containing herbicides are applied. Glyphosate containing herbicides are normally only applied in an agricultural environment. Therefore, glyphosate tolerance will not provide a selective advantage under natural conditions. In addition, other herbicides will remain available that could be used to control glyphosate tolerant beet species, such as weed beets.⁵ These other herbicides cannot be used in a sugar beet crop, because sugar beets are also sensitive to them and would be destroyed. COGEM points out that this situation is comparable to the current situation, where conventional weed beets cannot be controlled when sugar beet is cultivated.

General surveillance

General surveillance has been introduced to be able to observe unexpected adverse effects of genetically modified crops on the environment. The setting or population in which these effects might occur is either not, or hardly predictable.

The central tool for general surveillance in case of cultivation of H7-1 sugar beet is an annual farm questionnaire which is addressed to a subset of farmers that cultivate sugar beet H7-1. In COGEM's view the questionnaire should not only contain questions about the performance of H7-1 sugar beet on the field, but should also contain questions about unusual effects of the H7-1 sugar beet on the whole of the farmer's premises. In addition, the questionnaire should be complemented with additional questions that could detect a change in the ability to establish feral populations. These questions could for example focus on susceptibility to diseases and/or pests, effects of abiotic stress conditions and changes in the number of observed volunteers. Although the farm questionnaire contains questions on disease and pest susceptibility, questions on the effect of abiotic stress conditions and changes in the number of observed volunteers are missing.

COGEM is also of the opinion that the part of the farm questionnaire dealing with animals is too general. Birds, deer and insects are assigned to one category "wildlife". Information about the occurrence of wildlife should be obtained by different questions for specific groups of organisms (e.g. mammals, (predatory) birds, and insects). In addition, the farmer should be asked whether unusual quantities of other animals were observed and whether dead animals were found. The questions in the farm questionnaire refer to the usual situation, but the usual situation is not well defined. It would be better to rephrase the questions to acquire data that can be used to detect negative or positive trends in populations of organisms relevant to the monitoring scheme.

Possibly, the glyphosate tolerance trait could be introduced into plants of other beet species, e.g. weed beets. In soil, weed beet seed may remain viable for a long period. The General Surveillance plan will be implemented for the duration of the authorization. No adverse effects are expected from the introduction of the glyphosate tolerant trait, but in order to allow detection of possible unforeseen delayed and/or indirect effects COGEM is of the opinion that General Surveillance should be carried out a prolonged period after sugar beet H7-1 cultivation. If sugar beet H7-1 is cultivated until the end of the authorization period, General Surveillance should not be terminated at the end of the authorization period.

Additional remark: non-target organisms

COGEM evaluated the studies that the applicant provided on the effect of H7-1 sugar beet on non-target organisms and concludes that the applicant did not conduct field experiments with sugar beet H7-1 to assess the effect of the gm-crop on non-target organisms. The applicant did refer to field experiments that were carried out with other glyphosate tolerant sugar beet crops. In COGEM's opinion field experiments that assess the effect of a gm-crop on non-target organisms should be carried out with the event for which the application is filed. The current application, however, concerns the cultivation of the glyphosate tolerant sugar beet H7-1. COGEM is of the opinion that studies on the effect of a gm-crop on non-target organisms are only necessary when it is likely that the expression product of the transgene could lead to an adverse effect on non-target organisms.⁹ It is unlikely that the glyphosate tolerance trait that is present in H7-1 sugar beet would directly affect non-target organisms. Therefore, studies on the effect of non-target organisms are not necessary for the current application.

Advice

COGEM has been asked to advice on the application for cultivation of sugar beet H7-1. Sugar beet volunteers are almost never formed outside the field, but may be formed in an agricultural environment. There are no indications that sugar beet can form feral populations outside an agricultural environment. In addition, there is no reason to assume that H7-1 sugar beet has an increased fitness or an increased potential to establish feral populations.

Sugar beet can fertilize several close relatives which are cultivated in the Netherlands. It may also fertilize wild sea beets which are rarely found in the coastal regions of the Netherlands. Therefore, the glyphosate tolerance trait can be introduced into other beet species. In addition, glyphosate tolerant weed beets may be formed.

Glyphosate tolerance will only provide a selective advantage in an agricultural environment and will not provide a selective advantage under natural conditions. In addition, other herbicides will remain available that could be used to control glyphosate tolerant beet species, e.g. weed beets. These other herbicides cannot be used in a sugar beet crop, because sugar beets are also sensitive to these herbicides. COGEM points out that this situation is comparable to the current situation, where conventional weed beets cannot be controlled when sugar beet is cultivated.

COGEM is of the opinion that the molecular characterization of H7-1 sugar beet contains shortcomings. A small unexplained fragment appears to be present in the Southern blot that was carried out to detect backbone fragments. Therefore, it is uncertain whether backbone fragments are absent in sugar beet H7-1. An explanation for the presence of this fragment should be provided.

In addition, the claim that the basepairs that flank the *cp4 epsps* expression cassette correspond to sugar beet genomic DNA is not substantiated with data. Therefore, it is not clear whether the sequences that flank the *cp4 epsps* expression cassette are sugar beet genomic DNA or whether other sequences have co-inserted with the expression cassette. The applicant should demonstrate that the sequences flanking the *cp4 epsps* expression cassette are sugar beet DNA. The applicant could do this by performing a PCR with primers that are located in the regions that flank the *cp4 epsps* expression cassette on non-transgenic sugar beet.

The provided BLAST analysis on the sequences spanning the junctions between the *cp4 epsps* expression cassette and its flanking regions originates from 2003. In addition, the applicant states that the sequence of the *cp4 epsps* expression cassette may not be used for analysis in non-secured databases. COGEM is of the opinion that it is unacceptable that an old BLAST analysis is provided and that the provided sequences may not be analysed.

COGEM also points out that the provided General Surveillance plan should be improved on several points. The farm questionnaire should be complemented with additional questions. In addition, in order to allow detection of possible unforeseen delayed and/or indirect effects General Surveillance should be carried out a prolonged period after sugar beet H7-1 cultivation. If sugar beet H7-1 is cultivated until the end of the authorization period, General Surveillance should not be terminated at the end of the authorization period.

Conclusion

As mentioned above, the file concerning cultivation of sugar beet H7-1 contains inadequacies. The applicant should provide information on the remaining issues before a decision on the authorization for cultivation of H7-1 sugar beet is taken by the competent authorities.

References

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