

Results of discussions at the Biodiversity Impact Assessment Committee

Name: Coleoptera pest resistant and herbicide glufosinate tolerant corn

(DvSSJ1, ipd072Aa, patch, Zea maize subsp. maize (L.) lltis)

γDP23211, OECD UI: DP-Ø23211-2γ

Contents of first class use, etc.:

Use, cultivation, processing, storage, transportation and disposal for food or feed, and acts incidental thereto.

Applicant: Corteva Agriscience Japan Co., Ltd.

Based on the biodiversity impact assessment report submitted by the applicant, the Crops Subcommittee will discuss the impact on biodiversity when using Type 1 use of this recombinant maize in accordance with the Type 1 Use Regulations. We considered the content of the applicant's evaluation. The main items confirmed are as follows.

#### 1 Regarding the results of biodiversity impact assessment

This recombinant maize has the target gene introduced through two-step sequence insertion. As a first step, we used the particle gun method to create an intermediate strain into which a landing pad (LP) sequence containing the target sequence of the recombinase FLP protein was introduced. In the second step, plasmid PHP74643, which was constructed based on plasmid pSB1 derived from *Agrobacterium* (*Agrobacterium tumefaciens*), was introduced by the *Agrobacterium* method, and T- The DNA region flanked by the target sequence of the FLP protein was replaced with the corresponding sequence in the LP sequence of the intermediate strain.

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This recombinant corn is

γ pmi gene encoding the PMI protein derived from *Escherichia coli*

γ pat gene encoding PAT protein derived from *Streptomyces viridochromogenes*

γ DvSSJ1 gene fragment derived from Western corn rootworm (*Diabrotica virgifera virgifera*) designed to produce double-stranded RNA (dsRNA) that causes RNA interference effects

γ ipd072Aa gene encoding IPD072Aa protein derived from *Pseudomonas chlororaphis*

According to the segregation pattern of the gene, it was confirmed that the expression cassette containing the expression cassette is integrated on the chromosome, and that one copy of the expression cassette is integrated in the LP sequence by Southern by sequence analysis and base sequence analysis using the Sanger method. Confirmed. Furthermore, it has been confirmed by Southern blot analysis that it has been stably transmitted over multiple generations. Furthermore, it was confirmed that the target gene or gene fragment was stably expressed over multiple generations by measuring the amount of RNA produced using the ELISA method for γ, γ, and γ, and by measuring the amount of produced RNA using the QuantiGene Plex 2.0 method for γ. , has been confirmed.

#### (1) Competitive advantage

Although corn has a long history of being cultivated in Japan, there have been no reports of it growing naturally in the natural environment.

This recombinant maize is endowed with resistance to Coleoptera pests due to DvSSJ1dsRNA and IPD072Aa protein, tolerance to the herbicide glufosinate due to PAT protein, and selection marker characteristics due to PMI protein, but all of these properties are associated with seed shedding and dormancy. It is not a trait related to etc. From this, it was thought that possessing these traits would not allow this recombinant maize to grow naturally in Japan's natural environment, and therefore would not increase its competitive advantage.

Based on the above, the applicant's conclusion that there is no risk that this recombinant maize will cause any impact on biodiversity due to its competitive advantage is judged to be appropriate.

#### (2) Productivity of harmful substances

Although corn has a long history of being cultivated in Japan, there have been no reports of corn producing harmful substances.

The effects of DvSSJ1dsRNA, IPD072Aa protein, PAT protein, and PMI protein produced by this recombinant maize are specific, and it was thought that they would not produce harmful substances by acting on the metabolic pathway of the host. Furthermore, no amino acid sequence similarity was observed between IPD072Aa protein, PAT protein, and PMI protein and known allergens.

In fact, when plowing tests and subsequent crop tests were conducted, no statistically significant differences were observed between this recombinant maize and the non-recombinant control maize in terms of germination rate and dry weight of radish. In addition, when soil microflora tests were conducted, no statistically significant differences were observed between this recombinant maize and the non-recombinant control maize in terms of the numbers of bacteria, actinomycetes, and filamentous bacteria.

Additionally, when the herbicide glufosinate is sprayed, N-acetyl-L-glufosinate is produced by the PAT protein. However, the toxicity of this metabolite to animals is lower than that of glufosinate, and under the Agricultural Chemicals Control Law, residue standards have been established for corn as one of the target compounds for glufosinate to be analyzed, and safe usage has been established through pesticide registration. Safety for humans, livestock and the environment is ensured.

On the other hand, the DvSSJ1dsRNA and IPD072Aa protein produced in this recombinant maize exhibit insecticidal activity against Coleoptera pests such as Western corn rootworm, but are not harmful to other wild animals and plants. Not yet. For this reason, four species of Coleoptera insects that live in Japan and are designated as endangered or near-threatened species have been identified as wild animals and plants that cannot be denied the possibility of being affected. However, it has been reported that the amount of pollen accumulated around corn fields is less than 10 grains/cm<sup>2</sup> when the distance is 10 m or more from the field, and it is also possible that the pollen or plants of this recombinant corn may be ingested. Since it is difficult to imagine that sexually active Coleoptera insects locally inhabit the vicinity of cultivation fields, it is extremely unlikely that the four identified Coleoptera insect species will be affected by this recombinant maize at the population level. It was considered.

Based on the above, the applicant's conclusion that there is no risk of this recombinant maize causing impacts on biodiversity due to its productivity of harmful substances is judged to be appropriate.

### (3) Hybridity

Maize can interbreed with related wild species of the genus *Teosinte* and *Tripsacum*, but these have not been reported to grow naturally in Japan. Therefore, wild animals and plants that may be affected by biodiversity due to the hybridity of this recombinant maize were not identified.

Based on the above, the applicant's conclusion that there is no risk that this recombinant maize will cause any impact on biodiversity due to hybridization is judged to be appropriate.

### 2 Conclusions of the Crops Subcommittee

Based on the above, we have determined that the conclusion of the biodiversity impact assessment report that there is no risk of impact on biodiversity in Japan when this recombinant corn is used in accordance with the Type 1 Usage Regulations is appropriate. did.