

Title

**Summary of the Literature Review for LLCotton25 cotton
July 1, 2022 – June 30, 2023**

Final ReportData or guideline requirement

Explanatory note on literature searching
conducted in the context of GMO applications for (renewed) market authorization
and annual post-market environmental monitoring reports on GMOs authorised in the EU market.
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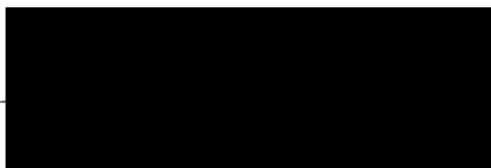
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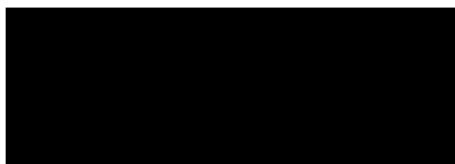
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Stage 1 assessment	
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Report	

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SUMMARY

The LLCotton25 cotton event was developed through *Agrobacterium*-mediated transformation using the vector pGSV71 containing the bar expression cassette. LLCotton25 cotton produces phosphinothricin acetyl transferase (PAT/*bar*) protein, which confers tolerance to glufosinate-ammonium herbicides. The OECD identifier is ACS-GHØØ1-3.

A scoping review was performed for the LLCotton25 cotton and its newly expressed protein, PAT/*bar*. The objective of this scoping review was to determine if there were studies about the molecular characterization of LLCotton25 cotton, its effect on food and feed safety or environmental safety, that might require in-depth examination. A set of broad literature searches was performed using several bibliographic databases covering scientific literature from July 1, 2022 to June 30, 2023. Additional sources of information, such as web pages of food safety, agriculture, and biotechnology-related authorities were searched for the same time window, along with the bibliographies of relevant reviews. The references identified were evaluated for potential relevance to the scoping review questions according to pre-defined criteria.

These literature searches identified a total of 63 unique publications, which were subject to rapid assessment to exclude obviously irrelevant publications. One publication was progressed for detailed assessment and determined to be not relevant after detailed review.

No new publications were found that contained new data on the molecular characterization of the LLCotton25 cotton and its newly expressed protein, PAT/*bar*. Similarly, no new publications were found that suggested any potential adverse effects of LLCotton25 cotton on human health, animal health, or the environment. No issues or topics were identified that would trigger or warrant more specific question formulation or indicate that a systematic review would be of value.

1. INTRODUCTION

The LLCotton25 cotton event was developed through *Agrobacterium*-mediated transformation using the vector pGSV71 containing the bar expression cassette. LLCotton25 cotton produces phosphinothricin acetyl transferase (PAT/*bar*) protein, which confers tolerance to glufosinate-ammonium herbicides. The OECD identifier is ACS-GHØØ1-3.

The objective of the literature searches described here was to determine if there were publications published between July 1, 2022 and June 30, 2023 that mention the molecular characterization of the LLCotton25 cotton, and/or any adverse effect of LLCotton25 cotton in food, feed or the environment. In that context, a broad and inclusive literature search was performed, and the articles retrieved were reviewed in a comprehensive and transparent manner. This was intended as a scoping review. The literature review was performed as recommended in the European Food Safety Authority (EFSA) explanatory note on literature searching conducted in the context of Genetically Modified Organisms (GMO; (1)) applications and post-market environmental monitoring activities (2019).

The literature searches were performed for the LLCotton25 cotton and its newly expressed proteins, PAT/*bar*. The search terms also included relevant synonyms, the trade name and intended trait. When needed, plant species and general GMO terms were used to limit the search results (described in Section 3.3).

2. OVERALL METHODS

2.1. Objective of the scoping review

The objective of the scoping review was to survey the evidence base for the LLCotton25 cotton and its newly expressed protein, PAT/*bar*, in order to identify any specific issues related to food or feed safety, molecular characterization or environmental safety that might require in-depth examination.

2.2. Review questions

Review questions were formulated to conform to PE(I)CO structure (Population, Exposure (Intervention), Comparators, Outcome) if possible, and to address data requirements. They were modeled after the review question examples provided in the EFSA 2019 explanatory note (1).

Question 1: Were any studies published during the reporting period that describe adverse effects on human or animal health or the environment of the LLCotton25 cotton and its newly expressed proteins PAT/*bar*?

Key elements:

Population: Human health; animal health; environmental safety

Exposure: LLCotton25 cotton, derived food/feed products, newly expressed proteins in LLCotton25 cotton

Comparators: When applicable, comparable populations or subjects exposed to appropriate controls (e.g., vehicle only, innocuous control protein, non-GM comparator) or conventional counterpart used for comparative analysis of plant material

Outcome: Adverse effects

Question 2: Were any studies published during the reporting period that focus on molecular characterization of the LLCotton25 cotton and its newly expressed proteins PAT/*bar* in cotton?

Key elements:

Population: LLCotton25 cotton and newly expressed proteins in LLCotton25 cotton

Outcome: Molecular characterization (which would indicate the information/data requirement for molecular characteristics)

2.3. Criteria for relevance

Criteria for establishing the relevance of retrieved publications were defined prior to conduct of the search. These criteria were modeled after those given in the EFSA 2019 explanatory note (1) and are described in Table 1.

Table 1: Eligibility/inclusion criteria to establish the relevance of retrieved publications

Concepts	Criteria	Comment
Key elements of review questions with PECO structure		
Population	The publication addresses human and animal health, and/or the environment (including biodiversity, ecosystem services, service providing units, and endangered species) as general protection goals	From the publications that address the GMO under consideration, those that address protection goals relevant to the risk assessment of the GMO are eligible
Exposure (Intervention)	The publication addresses the GMO, derived food/feed products, and/or the intended trait(s) (e.g., newly expressed proteins(s)) that are identical or like those under regulatory review	This enables the selection of publications that address the GMO, derived food/feed products, and/or the intended trait(s) under consideration
Comparator	If the publication reports a comparative study that uses plant material as test material, eligible publications must report a non-GM variety as comparator	In those cases where the publication addresses the GMO under consideration, reports a comparative analysis study and uses plant material as test material, eligible publications also need to include an appropriate non-GM line as comparator
Outcome	The publication addresses effects/impacts on human and animal health, and/or the environment	Publications that address the GMO under consideration also need to address effects/impacts on entities of concern, and potential determinants of exposure that place these entities at risk, in order to be relevant to the risk assessment of the GMO

Additional concepts		
Information/data requirements	The publication reports information pertaining to one or more information/data requirement(s) outlined in Appendix A for the GMO and derived food/feed products under consideration, including the intended trait(s)	Publications that potentially contribute to the knowledge informing the risk assessment of the GMO under consideration, and thus the risk hypotheses addressed, taking account of both hazard and exposure, can be considered relevant according to this eligibility/inclusion criterion. Publications addressing other issues such as benefits, socio-economics, ethics, crop protection, detection methods, efficacy, public perception and risk communication can be excluded, as they are not necessarily relevant to the risk assessment of GMOs
Plant species	The publication addresses the same plant species as the GMO under consideration	This eligibility/inclusion criterion permits the exclusion of publications on GMOs that contain the same intended trait(s) as the GMO under consideration, but which are introduced in another plant species
Scope of GMO application	The publication addresses pathways and levels of exposure to the GMO, derived food/feed products, and the intended trait(s) that are relevant for the intended uses of the GMO and derived food/feed products under regulatory review	From the publications that address the GMO under consideration, those that consider pathways and levels of exposure relevant to the scope of the GMO application (i.e., import and processing for food/feed uses, cultivation) are eligible
Target pests/organisms	The publication addresses target pests/organisms that are established in the EU	This permits the exclusion of publications that address interactions between the GMO and target pests/organisms that do not occur in the EU

Stacked events obtained by conventional crosses/ subcombinations	The publication addresses the higher stacked event and/or a subcombination or subcombinations of the single events of the higher stacked event, independently of its/their origin	This permits the selection of publications on the higher stacked event and/or subcombinations of the single events of the higher stacked event that are in the scope of the GMO application(s), independently of their origin. This permits the exclusion of publications on the single events of the higher stacked event, because the risk assessment of GMO applications for stacked events covers only the products in the scope of the GMO application – i.e., the higher stacked event and subcombinations of the singles involved, independently of their origin
Molecular stacks	The publication addresses: the molecular stack; all newly expressed proteins in the molecular stack; and/or one or several of the newly expressed proteins in the molecular stack that has/have not been previously risk assessed by EFSA and/or its GMO Panel and for which no safe use has been determined yet by EFSA and/or its GMO Panel	This permits the exclusion of publications that address one or several (not all) of the newly expressed proteins in the molecular stack that has/have been previously risk assessed by EFSA and/or its GMO Panel and for which the safe use has been determined by EFSA and/or its GMO Panel
Previously risk assessed publications	The publication has not been previously risk assessed by EFSA and/or its GMO Panel and is not cited/referenced in an EFSA/GMO Panel output	This permits the exclusion of publications that have been previously risk assessed by EFSA and/or its GMO Panel and cited/referenced in an EFSA/GMO Panel output
Access	Full-text document is accessible	If potentially relevant full-text documents cannot be obtained, they should be listed in a table with a description of the (unsuccessful) methods that have been used to try to obtain a copy

Reporting format	The publication presents original/primary data, or it is a risk assessment from a relevant key organisation (such as regulatory agencies and risk assessment bodies involved in the risk assessment of GMOs)	This permits the exclusion of publications that do not present original/primary data (e.g., editorials, position papers), and the inclusion of relevant risk assessments performed and reported by relevant key organisations. Reviews should only be included if they present data that are not available from a primary research study
Reporting format	A study in a publication should only be presented once, but if it is presented in more than one publication, all publications should be listed and grouped	Duplicate publications should be excluded at the screening stage. Only one copy of a study is required even if it is reported in different publications, and identified in more than one database

Table adapted from EFSA, 2019: Explanatory note on literature searching conducted in the context of GMO applications for (renewed) market authorisation and annual post-market environmental monitoring reports on GMOs authorised in the EU market (1).

2.4. Reference publication

A publication that is relevant for LLCotton25 cotton was known before starting the search, and was used as reference to test the search profile:

- Naegeli, H., Bresson, J.-L., Dalmay, T., Dewhurst, I.C., Epstein, M.M., Firbank, L.G., Guerche, P., Hejatko, J., Moreno, F.J., Mullins, E., Nogué, F., Rostoks, N., Sánchez Serrano, J.J., Savoini, G., Veromann, E., Veronesi, F., Álvarez, F., Ardizzone, M., Paraskevopoulos, K. (2018). Assessment of genetically modified LLCotton25 for renewal of authorisation under Regulation (EC) No 1829/2003 (application EFSA-GMO-RX-010). *EFSA Journal* 16(11) e05473

Since this reference was published in 2018 (before the current search period), the search profile was tested without limiting it to documents updated during the search period (UP>=20220701 and UP<=20230630).

3. SEARCH METHODS AND OUTCOMES

The search strategies used here followed the 2019 EFSA explanatory note on literature searching conducted in the context of GMO applications and post-market environmental monitoring activities (1). The search strategies were designed to be broad and sensitive enough to capture any relevant publications, if available.

An information specialist with background in plant biotechnology selected the databases, identified relevant search terms, developed search profiles, designed search strategies, and conducted the searches.

3.1. Time window and date of the literature search

The database searches were performed on July 4, 2023. Only documents updated between July 1, 2022 and June 30, 2023, were considered in the search. The dates of most recent database updates are provided in [Table 4](#).

3.2. Databases used in the literature search

All searches were performed in the host STN (Scientific and Technical Information Network), an online database service operated jointly by CAS and FIZ Karlsruhe. STN provides access to a broad range of databases from the most renowned database producers worldwide.

The searches described here were performed in five databases: three multidisciplinary/large databases (Biosis, Medline and CA-Plus) and two subject-specific databases focused on agriculture-related topics (Agricola and CABA).

See [Appendix 1](#) for detailed database descriptions.

3.3. Search strategy

The search profile was designed to cover event name, trade name, newly expressed protein, and intended trait. Since the 'newly expressed proteins' profiles and the 'intended trait' profiles produced too many results when used on their own, they were combined with additional profiles: the 'newly expressed protein' profile were combined with a 'plant species' profile while the 'intended trait' profiles were combined with a 'general GMO' profile as well as with the 'plant species' profile. The reference publication ([Section 2.4](#)) was identified by the search profiles confirming the validity of the applied search strategy. See Table 2 for a detailed search profile.

Table 2: Search profile for database search

Set	Search string	Concepts
1	s LLCotton25 or LLCotton(w)25 or LL(w)cotton25 or LL(w)cotton(w)25 or ACS-GH001-3 or ACS(w)GH001(w)3 or ACSGH001(w)3 or ACS-GH001-3 or ACS(w)GH001(w)3 or ACSGH001(w)3	Event name
2	libertylink or libertylinktm or libertylinkrtm or liberty(w)link or liberty(w)linktm or liberty(w)linkrtm or LL or LLTM or LLRTM	Trade name
3	((bar or pat)(2a)(gene# or protein# or enzyme#)) or ppt(2w)acetyltransferase or ppt(2w)acetyl(w)transferase or pt(w)n(2w)acetyltransferase or pt(w)n(2w)acetyl(w)transferase or phosphinothricin(w)n(w)acetyltransferase or phosphinothricin(2w)acetyltransferase or phosphinothricin(2w)acetyl(w)transferase or phosphinothricinacetyl(w)transferase	Newly expressed protein
4	(herbicide? or bialaphos or basta or glufosinate or gluphosinate or phosphinothricin or liberty?)(5a)(resist? OR protect? OR toleran?)	Intended trait
5	cotton# or gossypium or G(w)hirsutum or g(w)barbadense	Plant species

6	GMO OR GMOs OR LMO OR LMOs OR GM OR GE OR transgen? OR (genetic?(3a)(modif? OR transform? OR manipulat? OR improv? OR engineer?))	GMO general
7	(2 or 3) and 5	(Trade name or Newly expressed protein) AND Plant species
8	4 and 5 and 6	Intended trait AND Plant species AND GMO general
9	1 or 7 or 9	Event name OR ((Trade name OR Newly expressed protein) AND Plant species) OR (Intended trait AND Plant species AND GMO general)

All searches were performed in the Basic Index (BI) field, which includes the following subject headings/field names:

- **Agricola:** title (TI), controlled term (CT), supplementary term (ST), abstract (AB), named person (NA), corporate name (CO), note (NTE), geographic term, CABA and other fields (GT)
- **Biosis:** title (TI), abstract (AB), biosystematic codes (BC), chemical name (CN), controlled term (CT), gene name (GEN), geographic term (GT), organism (ORGN) and supplementary term (ST); as well as CAS Registry Numbers (RN)
- **CA-Plus:** title (TI), supplementary term (ST), index term (IT) and abstract (AB); as well as CAS Registry Numbers
- **CABA:** title (TI), controlled term (CT), supplementary term (ST), broader term (BT), abstract (AB), organism name (ORGN) and geographic term (GT); as well as CAS Registry Numbers
- **Medline:** title (TI), chemical name (CN), gene name (GEN), controlled term (excluding MeSH numbers) (CT), supplementary term (ST), named person (NA), other source (OS), and abstract (AB), as well as CAS Registry Numbers and GenBank Numbers

Relevant controlled terms (Table 3) were not searched separately because they are included in the Basic Index and were captured by the free-text searches.

Table 3: Relevant controlled terms (CT) and index terms (IT) in each database

Database	Event	New proteins	Intended traits	Plant species	GM plants
Agricola	None	None	"HERBICIDE RESISTANCE"	"GOSSYPIMUM BARBADENSE" "GOSSYPIMUM HIRSUTUM"	"TRANSGENIC PLANTS"

Database	Event	New proteins	Intended traits	Plant species	GM plants
Biosis	None	None	No terms for herbicide resistance	COTTON	None
CABA	None	None	"HERBICIDE RESISTANCE"	"GOSSYPIMUM BARBADENSE" "GOSSYPIMUM HIRSUTUM"	"TRANSGENIC PLANTS"
CAS	None	None	"HERBICIDE RESISTANCE"	"GOSSYPIMUM BARBADENSE"/CT "GOSSYPIMUM HIRSUTUM"/CT	"GENETICALLY MODIFIED PLANTS"
Medline	None	None	"HERBICIDE RESISTANCE"	GOSSYPIMUM/CT	"PLANTS, GENETICALLY MODIFIED"

The search results were limited to documents updated between July 1, 2022 and June 30, 2023 (UP>=20220701 and UP<=20230630), and to non-patent documents (not P/DT). To ensure that documents with indexing errors where two document types (DTs) (one eligible and one ineligible) were attached to a single record were not missed, documents with both 'journal' and 'patent' as document type were also kept. These putative documents would be identified with (P/DT AND J/DT) in CABA and CAPlus.

Table 4 summarizes the number of results obtained from each of the databases searched.

See [Appendix 2](#) for a complete search history.

Table 4: Overview of the selected databases and summary of search results from each database

Database	AGRICOLA	BIOSIS	CABA	CA-Plus	Medline
Database Provider	STN International	STN International	STN International	STN International	STN International
Coverage	1970-present	1926-present	1973-present	1907-present	1946-present
Date of search	4 Jul 2023	4 Jul 2023	4 Jul 2023	4 Jul 2023	4 Jul 2023
Datespan of the search	1 Jul 2022 – 30 Jun 2023	1 Jul 2022 – 30 Jun 2023	1 Jul 2022 – 30 Jun 2023	1 Jul 2022 – 30 Jun 2023	1 Jul 2022 – 30 Jun 2023
Latest database update	7 Jun 2023	28 Jun 2023	27 Jun 2023	3 Jul 2023	3 Jul 2023
Number of records retrieved	4	19	31	15	8

Database	AGRICOLA	BIOSIS	CABA	CA-Plus	Medline
Number of records after duplicate removal	4	16	24	11	8
Number of relevant records after rapid assessment	0	0	1	0	0

4. INTERNET and MANUAL SEARCHES

4.1. Internet Searches of food safety, agriculture, and biotechnology-related authority webpages

A search of the web pages of food safety, agriculture, and biotechnology-related authorities was conducted. Search results were manually examined for relevant records that were either published during the time period under consideration (date span of search: July 1, 2022 to June 30, 2023) or refer to relevant records published during this time frame. Relevance of results were determined based on the criteria listed in Table 1 and they were summarized in Table 5. All web pages searched were justified by their recommendation in the EFSA 2019 explanatory note (1). Of the 13 key organisations cited in the EFSA 2019 explanatory note (1), Environment and Climate Change Canada and Intersecretarial Commission on Biosafety of GMOs (CIBIOGEM) were excluded, since they are not involved in the risk assessment of GM plants. The US-EPA website was excluded, since LLCotton25 cotton does not contain an insect-resistant trait. Therefore, the internet search was limited to 10 key organisations relevant for LLCotton25 cotton. Search terms consisted of LLCotton25 or LL25 or ACS-GHØØ1-3 and PAT/*bar* or Phosphinothricin (all searched singly, with no search limits applied).

Table 5: Results of search of food safety, agriculture, and biotechnology-related authority websites

Source Site Name	Website URL	Date of Most Recent Site Update (dd.mm.yyyy)	Date of Search (dd.mm.yyyy)	No. of Relevant Records
US Department of Agriculture (USDA)	https://www.usda.gov/	25.07.2023	25.07.2023	0
US Food and Drug Administration (FDA)	https://www.fda.gov/	25.07.2023	28.07.2023	0
Health Canada	https://www.canada.ca/en/health-canada.html	26.06.2023	10.07.2023	0
Canadian Food Inspection Agency (CFIA)	https://www.canada.ca/en/food-inspection-agency.html	26.06.2023	20.07.2023	0
Food Standards Australia New Zealand (FSANZ)	http://www.foodstandards.gov.au/Pages/default.aspx	30.06.2023	10.07.2023	0

Source Site Name	Website URL	Date of Most Recent Site Update (dd.mm.yyyy)	Date of Search (dd.mm.yyyy)	No. of Relevant Records
Office of the Gene Technology Regulator (OGTR) Australia	http://www.ogtr.gov.au/	06.04.2023	10.07.2023	0
National Technical Commission on Biosafety (CTNBio) Brazil	http://ctnbio.mcti.gov.br/en	24.07.2023	7/17/2023 to 7/24/2023	0
National Advisory Commission on Agricultural Biotechnology (CONABIA) Argentina	https://www.argentina.gob.ar/agroindustria/bioeconomia/biotechnologia	17.07.2023	17.07.2023	0
Genetic Engineering Approval Committee (GEAC) India	http://moef.gov.in/	19.07.2023	19.07.2023	0
Ministry of Agriculture, Forestry and Fisheries (MAFF) Japan	http://www.maff.go.jp/	27.07.2023	27.07.2023	0

4.2. Manual searches of reference lists of recent review articles

Recent review articles as sources of reference lists to search for potentially relevant studies were identified via searches of PubMed.gov for general terms such as “GMO” or “GM crops” in the titles and abstracts. The search of PubMed.gov was also restricted to recent reviews published between July 1, 2022 and June 30, 2023. The resulting number of relevant studies found within the bibliographies of these review articles is given in Table 6.

Table 6: Documents for which reference lists were scanned for relevant studies

No	Author(s) and Year	Title	Source	Number of relevant bibliographic references retrieved
1	Bhattacharjee S, Bhowmick R, Kant L, Paul K. 2023	Strategic transgene-free approaches of CRISPR-based genome editing in plants.	Mol Genet Genomics. 2023 May;298(3):507-520	0
2	Cermakova E, Lencova S, Mukherjee S, Horka P, Vobruba S, Demnerova K, Zdenkova K. 2023	Identification of Fish Species and Targeted Genetic Modifications Based on DNA Analysis: State of the Art.	Foods. 2023 Jan 3;12(1):228.	0

No	Author(s) and Year	Title	Source	Number of relevant bibliographic references retrieved
3	Connolly JB, Romeis J, Devos Y, Glandorf DCM, Turner G, Coulibaly MB. 2023	Gene drive in species complexes: defining target organisms.	Trends Biotechnol. 2023 Feb;41(2):154-164.	0
4	Eckerstorfer MF, Dolezel M, Engelhard M, Giovannelli V, Grabowski M, Heissenberger A, Lener M, Reichenbecher W, Simon S, Staiano G, Wüst Saucy AG, Zünd J, Lüthi C. 2023	Recommendations for the Assessment of Potential Environmental Effects of Genome-Editing Applications in Plants in the EU.	Plants (Basel). 2023 Apr 25;12(9):1764.	0
5	Ghidoli M, Ponzoni E, Araniti F, Miglio D, Pilu R. 2023	Genetic Improvement of <i>Camelina sativa</i> (L.) Crantz: Opportunities and Challenges.	Plants (Basel). 2023 Jan 27;12(3):570.	0
6	Krasnodębski C, Sawuła A, Kaźmierczak U, Żuk M. 2023	Oligo-Not Only for Silencing: Overlooked Potential for Multidirectional Action in Plants.	Int J Mol Sci. 2023 Feb 24;24(5):4466.	0
7	Křížkovská B, Viktorová J, Lipov J. 2022	Approved Genetically Modified Potatoes (<i>Solanum tuberosum</i>) for Improved Stress Resistance and Food Safety.	J Agric Food Chem. 2022 Sep 28;70(38):11833-11843.	0
8	Liang J, Yang X, Jiao Y, Wang D, Zhao Q, Sun Y, Li Y, Wu K. 2022	The evolution of China's regulation of agricultural biotechnology.	aBIOTECH. 2022 Dec 5;3(4):237-249.	0

No	Author(s) and Year	Title	Source	Number of relevant bibliographic references retrieved
9	Platani M, Sokefun O, Bassil E, Apidianakis Y. 2023	Genetic engineering and genome editing in plants, animals, and humans: Facts and myths. Gene.	2023 Mar 10; 856:147141.	0
10	Pott A, Bundschuh M, Otto M, Schulz R. 2023	Assessing Effects of Genetically Modified Plant Material on the Aquatic Environment Using higher-tier Studies.	Bull Environ Contam Toxicol. 2023 Jan 2;110(1):35.	0
11	Rai GK, Kumar P, Choudhary SM, Kosser R, Khanday DM, Choudhary S, Kumar B, Magotra I, Kumar RR, Ram C, Roupael Y, Corrado G, Behera TK. 2022	Biomimetic Strategies for Developing Abiotic Stress-Tolerant Tomato Cultivars:	An Overview. Plants (Basel). 2022 Dec 23;12(1):86	0
12	Rozas P, Kessi-Pérez EI, Martínez C. 2022	Genetically modified organisms: adapting regulatory frameworks for evolving genome editing technologies.	Biol Res. 2022 Oct 20;55(1):31.	0
13	Spök A, Sprink T, Allan AC, Yamaguchi T, Dayé C. 2022	Towards social acceptability of genome-edited plants in industrialised countries? Emerging evidence from Europe, United States, Canada, Australia, New Zealand, and Japan.	Front Genome Ed. 2022 Aug 31;4:899331.	0
14	Tatineni S, Hein GL. 2023	Plant Viruses of Agricultural Importance: Current and Future Perspectives of Virus Disease Management Strategies.	Phytopathology. 2023 Feb;113(2):117-141.	0

No	Author(s) and Year	Title	Source	Number of relevant bibliographic references retrieved
15	Tripathi S, Purchase D, Chandra R, Nadda AK, Bhargava PC. 2022	Mitigation of hazards and risks of emerging pollutants through innovative treatment techniques of post methanated distillery effluent - A review.	Chemosphere. 2022 Aug;300:134586 . doi: 10.1016/j.chemosphere.2022.134586.	0
16	Wang M, Wang H, Li K, Li X, Wang X, Wang Z. 2023	Review of CRISPR/Cas Systems on Detection of Nucleotide Sequences.	Foods. 2023 Jan 19;12(3):477.	0
17	Zimny T. 2023	Regulation of GMO field trials in the EU and new genomic techniques: will the planned reform facilitate experimenting with gene-edited plants?	BioTechnologia (Pozn). 2023 Mar 27;104(1):75-83.	0

5. RESULTS OF THE STUDY IDENTIFICATION AND SELECTION PROCESS

The database searches (Section 3) identified a total of 77 references, which were reduced to 63 references after removal of duplicates (Table 4). No additional studies were identified in the manual searches (Section 4).

5.1. Screening of titles and abstracts to exclude obviously irrelevant references (Stage 1)

All references identified in the database searches described in Section 3 were assessed for relevance based on information in their title and abstract by two reviewers independently. If opinions of relevance differed, the discrepancies were discussed between the reviewers and if a disagreement persisted, the publication under discussion was transferred to Stage 2 for detailed evaluation by the experts. In this search, both evaluators were in 100% agreement.

Clearly irrelevant records were tagged as “Not Relevant”. These included:

- Duplicated entries
- Secondary literature (reviews), other than assessments from regulatory agencies
- Articles on non-relevant topics like detection methods, socio-economic implications of GM crops, GM policy, agronomical performance, other herbicide tolerant GM crops, other insect resistant GM crops, unrelated topics, etc.

Publications which appeared to be relevant and those of unclear relevance were tagged as “Relevant” and progressed to Stage 2 (detailed assessment; see Section 5.2).

The number of publications excluded after rapid assessment for relevance is presented in Table 7 documenting the selection process.

5.2. Detailed assessment of eligible references (Stage 2)

Publications tagged as “Relevant” in Stage 1 were assessed in detail independently by two scientific experts in each of three corresponding areas (*i.e.*, Molecular Biology, Food and Feed Safety, Environmental Safety), based on the full text of the publications. If opinions of relevance differed between reviewers within each area, the initial reviewers discussed the discrepancy as necessary and consulted additional reviewers to resolve the discrepancy if needed.

In the relevance assessment of the literature review for the LLCotton25 cotton, reviewers agreed in 100% of the Stage 2 evaluations.

Table 7 gives an overview of the reference selection process and results of the detailed assessment.

Table 7: Results of the publication selection process

Total number of publications retrieved after all searches of the scientific literature (excluding duplicates)	63
Number of publications excluded from the search results after rapid assessment for relevance (Stage 1)	62
Total number of full-text documents assessed in detail	1
Number of publications excluded from further consideration after detailed assessment for relevance (Stage 2)	1
Total number of unobtainable/unclear publications	0
Total number of relevant publications	0

Table 8 lists the publications determined to be relevant based on the detailed evaluation. Publications that were clearly not relevant after a detailed assessment are listed in Table 9. Table 10 lists the publications for which full-text documents were unobtainable for detailed assessment or for which relevance was unclear after detailed assessment.

Table 8: Report of all relevant publications retrieved after detailed assessment of full-text documents for relevance: ordered by category of information/data requirement(s)

Main category of information/data requirement	Study (Author(s) and year)	Title	Source
No publications in any category.			

Table 9: Report of publications excluded from the risk assessment after detailed assessment of full-text documents

Study (Author(s) and year)	Title	Source	Reason(s) for exclusion based on eligibility/inclusion criteria listed in Table 1
Sharkey, S. M. Williams, B. J. Parker, K. M. 2021	Herbicide drift from genetically engineered herbicide -tolerant crops	Environmental Science + Technology (2021), Volume 55, Number 23, pp. 15559-15568, 107 refs. ISSN: 0013-936X; 1520-5851 DOI: https://doi.org/10.1021/acs.est.1c01906 .	The paper focuses on herbicide drift and the impacts of dicamba and 2,4-D on non-target vegetation in herbicide tolerant traits. The environmental safety assessment is not directly related to or relevant for LLCotton25 cotton.

Table 10: Report of unobtainable/unclear publications

Study (Author(s) and year)	Title	Source	Description of (unsuccessful) methods used to try and obtain a copy of the publication
No publications in this category.			

6. NARRATIVE SYNTHESIS/SUMMARY OF RELEVANT STUDIES

One publication was selected during Stage 1 evaluation (rapid assessment based on title and abstract). After Stage 2 evaluation (detailed review based on full text), it was determined that it was not relevant for the safety assessment of the LLCotton25 cotton and its newly expressed protein, PAT/*bar*.

7. CONCLUSION

The literature searches performed for the LLCotton25 cotton and its newly expressed proteins, PAT/*bar*, for the period from July 1, 2022 to June 30, 2023, identified a total of 63 unique publications (after duplicate removal). One publication was progressed for detailed assessment after excluding 62 obviously irrelevant publications during Stage 1 evaluation (rapid assessment based on title and abstract). The publication that progressed to Stage 2 was evaluated in detail, based on full text, for potential relevance, following the pre-established criteria listed in [Table 1](#).

No new publications were found that contained new data on the molecular characterization of the LLCotton25 cotton and its newly expressed protein, PAT/*bar*. Similarly, no new publications were found that suggested any potential adverse effects of LLCotton25 cotton on human health, animal health, or the environment. No issues or topics were identified that would trigger or warrant more specific question formulation or indicate that a systematic review would be of value.

8. REFERENCES

No.	Author(s), title, source, edition, year, pages
1.	Devos Y, Guajardo IM, Alvarez F and Glanville J. Explanatory note on literature searching conducted in the context of GMO applications for (renewed) market authorisation and annual post-market environmental monitoring reports on GMOs authorised in the EU market. EFSA supporting publications 2019:EN-1614. 62 pages. doi:10.2903/sp.efsa.2019.EN-1614.

9. APPENDICES

Appendix 1: Database descriptions

Host	File	Description
STN	AGRICOLA	<p>Agriculture Online Access is a bibliographic database containing selected worldwide literature of agriculture and related fields. AGRICOLA is the locator and bibliographic access and control system of the National Agricultural Library (NAL) collections and also includes records from other cooperating institutions. Coverage of the database includes agricultural economics and rural sociology, agricultural production, animal sciences, chemistry, entomology, food and human nutrition, forestry, natural resources, pesticides, plant science, soils and fertilizers, and water resources. Also covered are related areas such as biology and biotechnology, botany, ecology, and natural history.</p> <p>The database draws on bibliographies, serial articles, book chapters, monographs, computer files, serials, maps, audiovisuals, and reports. Bibliographic information, abstracts, geographic terms, controlled terms, and supplementary terms are searchable.</p>
STN	BIOSIS	<p>BIOSIS Previews® is the largest and most comprehensive life science database in the world. Amongst others subject coverage includes Agriculture, Biochemistry, Biophysics, Botany, Environmental Biology, Physiology, Toxicology.</p> <p>Sources include periodicals, journals, conference proceedings, reviews, reports, patents, and short communications. Nearly 6,000 life source journals, 1,500 international meetings as well as review articles, books, and monographs are reviewed for inclusion.</p> <p>Bibliographic information, indexing terms, abstracts, and CAS Registry Numbers are all searchable.</p>
STN	CABA/CAB	<p>The CAB Abstracts database covers worldwide literature from all areas of agriculture and related sciences including Agriculture, Agricultural chemicals, Animal sciences and production, Crop protection, Crop sciences and production, Environment, Soils and fertilizers.</p> <p>Sources for CABA include journals, books, reports, published theses, conference proceedings, and patents.</p> <p>Bibliographic information, indexing terms, abstracts, and CAS Registry Numbers are searchable.</p>
STN	CAS/CA-PLUS	<p>The Chemical Abstracts (CA) database covers all areas of Biochemistry, Chemistry and Chemical engineering, and related sciences.</p> <p>Sources include over 8,000 journals, patents from 38 national patent offices and two international patent organizations, technical reports, books, conference proceedings, and dissertations. Electronic only journals and Web preprints are also covered.</p> <p>Bibliographic terms, indexing terms, roles, CAS Registry Numbers, International Patent Classification, and abstracts are searchable.</p>

Host	File	Description
STN	MEDLINE	<p>MEDLINE contains information on every area of medicine. The MEDLINE database corresponds to Index Medicus, Index to Dental Literature, and International Nursing Index; OLDMEDLINE, with data from NLM's from the Cumulated Index Medicus (1960-1965) and Current List of Medical Literature (1958-1959); and, since August 2001, IN-PROCESS records, the latest documents before they have been completely indexed for inclusion on MEDLINE.</p> <p>Sources include journals and chapters in books or symposia. Bibliographic information, indexing terms, abstracts, chemical names, and CAS Registry Numbers are all searchable.</p> <p>Online thesauri are available for the Medical Subject Headings (/MN), Controlled Terms (/CT) and Chemical Name (/CN) fields.</p>

Appendix 2: Search history

FILE 'MEDLINE' ENTERED AT 07:45:23 ON 04 JUL 2023

L1 3 SEA LLCOTTON25 OR LLCOTTON(W)25 OR LL(W)COTTON25 OR LL(W)COTTON
(W)25 OR ACS-GH001-3 OR ACS(W)GH001(W)3 OR ACSGH001(W)3 OR
ACS-GH001-3 OR ACS(W)GH001(W)3 OR ACSGH001(W)3

L2 15587 SEA LIBERTYLINK OR LIBERTYLINKTM OR LIBERTYLINKRTM OR LIBERTY(W)
)LINK OR LIBERTY(W)LINKTM OR LIBERTY(W)LINKRTM OR LL OR LLTM
OR LLRTM

L3 1589 SEA ((BAR OR PAT) (2A) (GENE# OR PROTEIN# OR ENZYME#)) OR
PPT(2W)ACETYLTRANSFERASE OR PPT(2W)ACETYL(W)TRANSFERASE OR
PT(W)N(2W)ACETYLTRANSFERASE OR PT(W)N(2W)ACETYL(W)TRANSFERASE

L4 208 SEA PHOSPHINOTHRICIN(W)N(W)ACETYLTRANSFERASE OR PHOSPHINOTHRICI
N(2W)ACETYLTRANSFERASE OR PHOSPHINOTHRICIN(2W)ACETYL(W)TRANSFER
ASE OR PHOSPHINOTHRICINACETYL(W)TRANSFERASE

L5 1664 SEA (L3 OR L4)

L6 3816 SEA (HERBICID? OR BIALAPHOS OR BASTA OR GLUFOSINATE OR
GLUPHOSINATE OR PHOSPHINOTHRICIN OR LIBERTY?) (5A) (RESIST? OR
PROTECT? OR TOLERAN?)

L7 30428 SEA COTTON# OR GOSSYPIUM OR G(W)HIRSUTUM OR G(W)BARBADENSE

L8 4174968 SEA GMO OR GMOS OR LMO OR LMOS OR GM OR GE OR TRANSGEN? OR
(GENETIC?(3A) (MODIF? OR TRANSFORM? OR MANIPULAT? OR IMPROV? OR
ENGINEER?))

L9 29 SEA (L2 OR L5) AND L7

L10 119 SEA L6 AND L7 AND L8

L11 141 SEA L1 OR L9 OR L10

L12 21 SEA L11 AND PY>=2021

L13 8 SEA L12 AND UP>=20220701 AND UP<=20230630

FILE 'BIOSIS' ENTERED AT 07:45:28 ON 04 JUL 2023

L14 5 SEA LLCOTTON25 OR LLCOTTON(W)25 OR LL(W)COTTON25 OR LL(W)COTTON
(W)25 OR ACS-GH001-3 OR ACS(W)GH001(W)3 OR ACSGH001(W)3 OR
ACS-GH001-3 OR ACS(W)GH001(W)3 OR ACSGH001(W)3

L15 15839 SEA LIBERTYLINK OR LIBERTYLINKTM OR LIBERTYLINKRTM OR LIBERTY(W)
)LINK OR LIBERTY(W)LINKTM OR LIBERTY(W)LINKRTM OR LL OR LLTM
OR LLRTM

L16 2985 SEA ((BAR OR PAT) (2A) (GENE# OR PROTEIN# OR ENZYME#)) OR
PPT(2W)ACETYLTRANSFERASE OR PPT(2W)ACETYL(W)TRANSFERASE OR
PT(W)N(2W)ACETYLTRANSFERASE OR PT(W)N(2W)ACETYL(W)TRANSFERASE

L17 337 SEA PHOSPHINOTHRICIN(W)N(W)ACETYLTRANSFERASE OR PHOSPHINOTHRICI
N(2W)ACETYLTRANSFERASE OR PHOSPHINOTHRICIN(2W)ACETYL(W)TRANSFER
ASE OR PHOSPHINOTHRICINACETYL(W)TRANSFERASE

L18 3092 SEA (L16 OR L17)

L19 10568 SEA (HERBICID? OR BIALAPHOS OR BASTA OR GLUFOSINATE OR
GLUPHOSINATE OR PHOSPHINOTHRICIN OR LIBERTY?) (5A) (RESIST? OR
PROTECT? OR TOLERAN?)

L20 79314 SEA COTTON# OR GOSSYPIUM OR G(W)HIRSUTUM OR G(W)BARBADENSE

L21 494793 SEA GMO OR GMOS OR LMO OR LMOS OR GM OR GE OR TRANSGEN? OR
(GENETIC?(3A) (MODIF? OR TRANSFORM? OR MANIPULAT? OR IMPROV? OR
ENGINEER?))

L22 90 SEA (L15 OR L18) AND L20

L23 270 SEA L19 AND L20 AND L21

L24 347 SEA L14 OR L22 OR L23

L25 32 SEA L24 AND PY>=2021

L26 19 SEA L25 AND UP>=20220701 AND UP<=20230630

FILE 'AGRICOLA' ENTERED AT 07:45:33 ON 04 JUL 2023

L27 2 SEA LLCOTTON25 OR LLCOTTON(W)25 OR LL(W)COTTON25 OR LL(W)COTTON
(W)25 OR ACS-GH001-3 OR ACS(W)GH001(W)3 OR ACSGH001(W)3 OR
ACS-GH001-3 OR ACS(W)GH001(W)3 OR ACSGH001(W)3

L28 3677 SEA LIBERTYLINK OR LIBERTYLINKTM OR LIBERTYLINKRTM OR LIBERTY(W)
)LINK OR LIBERTY(W)LINKTM OR LIBERTY(W)LINKRTM OR LL OR LLTM
OR LLRTM

L29 826 SEA ((BAR OR PAT) (2A) (GENE# OR PROTEIN# OR ENZYME#)) OR
PPT(2W)ACETYLTRANSFERASE OR PPT(2W)ACETYL(W)TRANSFERASE OR
PT(W)N(2W)ACETYLTRANSFERASE OR PT(W)N(2W)ACETYL(W)TRANSFERASE
L30 257 SEA PHOSPHINOTHRICIN(W)N(W)ACETYLTRANSFERASE OR PHOSPHINOTHRICI
N(2W)ACETYLTRANSFERASE OR PHOSPHINOTHRICIN(2W)ACETYL(W)TRANSFER
ASE OR PHOSPHINOTHRICINACETYL(W)TRANSFERASE
L31 908 SEA (L29 OR L30)
L32 8861 SEA (HERBICID? OR BIALAPHOS OR BASTA OR GLUFOSINATE OR
GLUPHOSINATE OR PHOSPHINOTHRICIN OR LIBERTY?) (5A) (RESIST? OR
PROTECT? OR TOLERAN?)
L33 67931 SEA COTTON# OR GOSSYPIMUM OR G(W)HIRSUTUM OR G(W)BARBADENSE
L34 113118 SEA GMO OR GMOS OR LMO OR LMOS OR GM OR GE OR TRANSGEN? OR
(GENETIC?(3A) (MODIF? OR TRANSFORM? OR MANIPULAT? OR IMPROV? OR
ENGINEER?))
L35 53 SEA (L28 OR L31) AND L33
L36 234 SEA L32 AND L33 AND L34
L37 276 SEA L27 OR L35 OR L36
L38 14 SEA L37 AND PY>=2021
L39 4 SEA L38 AND UP>=20220701 AND UP<=20230630

FILE 'CABA' ENTERED AT 07:45:38 ON 04 JUL 2023

L40 5 SEA LLCOTTON25 OR LLCOTTON(W)25 OR LL(W)COTTON25 OR LL(W)COTTON
(W)25 OR ACS-GH001-3 OR ACS(W)GH001(W)3 OR ACSGH001(W)3 OR
ACS-GH001-3 OR ACS(W)GH001(W)3 OR ACSGH001(W)3
L41 6044 SEA LIBERTYLINK OR LIBERTYLINKTM OR LIBERTYLINKRTM OR LIBERTY(W
)LINK OR LIBERTY(W)LINKTM OR LIBERTY(W)LINKRTM OR LL OR LLTM
OR LLRTM
L42 1630 SEA ((BAR OR PAT) (2A) (GENE# OR PROTEIN# OR ENZYME#)) OR
PPT(2W)ACETYLTRANSFERASE OR PPT(2W)ACETYL(W)TRANSFERASE OR
PT(W)N(2W)ACETYLTRANSFERASE OR PT(W)N(2W)ACETYL(W)TRANSFERASE
L43 387 SEA PHOSPHINOTHRICIN(W)N(W)ACETYLTRANSFERASE OR PHOSPHINOTHRICI
N(2W)ACETYLTRANSFERASE OR PHOSPHINOTHRICIN(2W)ACETYL(W)TRANSFER
ASE OR PHOSPHINOTHRICINACETYL(W)TRANSFERASE
L44 1736 SEA (L42 OR L43)
L45 19754 SEA (HERBICID? OR BIALAPHOS OR BASTA OR GLUFOSINATE OR
GLUPHOSINATE OR PHOSPHINOTHRICIN OR LIBERTY?) (5A) (RESIST? OR
PROTECT? OR TOLERAN?)
L46 112536 SEA COTTON# OR GOSSYPIMUM OR G(W)HIRSUTUM OR G(W)BARBADENSE
L47 204631 SEA GMO OR GMOS OR LMO OR LMOS OR GM OR GE OR TRANSGEN? OR
(GENETIC?(3A) (MODIF? OR TRANSFORM? OR MANIPULAT? OR IMPROV? OR
ENGINEER?))
L48 103 SEA (L41 OR L44) AND L46
L49 524 SEA L45 AND L46 AND L47
L50 598 SEA L40 OR L48 OR L49
L51 44 SEA L50 AND PY>=2021
L52 31 SEA L51 AND UP>=20220701 AND UP<=20230630
L53 31 SEA L52 NOT P/DT
L54 0 SEA L52 AND (P/DT AND J/DT)
L55 31 SEA L53 OR L54

FILE 'HCAPLUS' ENTERED AT 07:45:46 ON 04 JUL 2023

L56 11 SEA LLCOTTON25 OR LLCOTTON(W)25 OR LL(W)COTTON25 OR LL(W)COTTON
(W)25 OR ACS-GH001-3 OR ACS(W)GH001(W)3 OR ACSGH001(W)3 OR
ACS-GH001-3 OR ACS(W)GH001(W)3 OR ACSGH001(W)3
L57 22171 SEA LIBERTYLINK OR LIBERTYLINKTM OR LIBERTYLINKRTM OR LIBERTY(W
)LINK OR LIBERTY(W)LINKTM OR LIBERTY(W)LINKRTM OR LL OR LLTM
OR LLRTM
L58 5695 SEA ((BAR OR PAT) (2A) (GENE# OR PROTEIN# OR ENZYME#)) OR
PPT(2W)ACETYLTRANSFERASE OR PPT(2W)ACETYL(W)TRANSFERASE OR
PT(W)N(2W)ACETYLTRANSFERASE OR PT(W)N(2W)ACETYL(W)TRANSFERASE
L59 809 SEA PHOSPHINOTHRICIN(W)N(W)ACETYLTRANSFERASE OR PHOSPHINOTHRICI
N(2W)ACETYLTRANSFERASE OR PHOSPHINOTHRICIN(2W)ACETYL(W)TRANSFER
ASE OR PHOSPHINOTHRICINACETYL(W)TRANSFERASE

L60 6012 SEA (L58 OR L59)
L61 30936 SEA (HERBICID? OR BIALAPHOS OR BASTA OR GLUFOSINATE OR
 GLUPHOSINATE OR PHOSPHINOTHRICIN OR LIBERTY?) (5A) (RESIST? OR
 PROTECT? OR TOLERAN?)
L62 293160 SEA COTTON# OR GOSSYPIUM OR G(W)HIRSUTUM OR G(W)BARBADENSE
L63 746613 SEA GMO OR GMOS OR LMO OR LMOS OR GM OR GE OR TRANSGEN? OR
 (GENETIC?(3A) (MODIF? OR TRANSFORM? OR MANIPULAT? OR IMPROV? OR
 ENGINEER?))
L64 304 SEA (L57 OR L60) AND L62
L65 1397 SEA L61 AND L62 AND L63
L66 1587 SEA L56 OR L64 OR L65
L67 366 SEA L66 AND PY>=2021
L68 74 SEA L67 AND UP>=20220701 AND UP<=20230630
L69 15 SEA L68 NOT P/DT
L70 0 SEA L68 AND (P/DT OAND J/DT)
L71 15 SEA L69 OR L70

FILE 'MEDLINE, BIOSIS, AGRICOLA, CABA, HCAPLUS' ENTERED AT 01:45:51 ON 04
JUL 2023

L72 63 DUP REM L13 L26 L39 L55 L71 (14 DUPLICATES REMOVED)
 ANSWERS '1-8' FROM FILE MEDLINE
 ANSWERS '9-24' FROM FILE BIOSIS
 ANSWERS '25-28' FROM FILE AGRICOLA
 ANSWERS '29-52' FROM FILE CABA
 ANSWERS '53-63' FROM FILE HCAPLUS