

First Latin American off-patent corn event - Fenaltec 22

Jenny Paola Jiménez-Barreto^{1,2,3*}, José Ever Vargas Sanchez²,
Julian Mora-Oberlaender¹ and Alejandro Chaparro-Giraldo¹

Crop Breeding and Applied Biotechnology
24(2): e46582428, 2024
Brazilian Society of Plant Breeding.
Printed in Brazil
<http://dx.doi.org/10.1590/1984-70332024v24n2n21>

Abstract: Genetically modified (GM) crops have been on the market for almost 27 years and since the beginning have been protected by intellectual property (IP) rights that restrict their use and commercialization by third parties. In addition, their development is typically associated with elevated costs, making their production by public research institutions extremely difficult, especially in developing countries. Nevertheless, many patents for the first generation of GM crops have already expired, and others will do so soon, opening the path to generic GM crops. Using technologies in the public domain makes it possible to deliver GM seeds adapted to the local environment at affordable prices. This paper describes the development of the first Latin American off-patent GM corn and discusses the relevant IP and regulatory issues that allowed its commercial release in Colombia. The approach exposed here can be utilized for other crops or characteristics of agronomic interest.

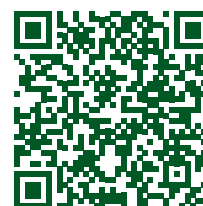
Keywords: Off-patent event, generic GM crops, GM crop biosafety, GM crop regulation, freedom to operate

INTRODUCTION


For more than 20 years, transgenic crops have been essential tools for improving productivity and reducing the economic and environmental costs of agriculture (Brookes and Barfoot 2020, Brookes and Barfoot 2022).

In Colombia, genetically modified (GM) corn, cotton, and blue carnation crops are cultivated. This country has developed technical capacities both for the research and development of plant biotechnology through research centers and universities and from a regulatory standpoint (Mora-Oberlaender et al. 2018). Nevertheless, until now, only one locally developed biotech crop has been released for commercialization: TC1507 off-patent corn Fenaltec 22.

One of the reasons for this situation is the elevated cost of developing a commercial biotech crop. In addition to the use of specialized facilities, such as laboratories and greenhouses, biotech crops must comply with a regulatory framework that can be very expensive. McDougall (2011) estimated the average cost for the development of a new transgenic plant event by a multinational corporation, from gene discovery to commercial liberation, at US \$136 million. Nonetheless, Schiek et al. (2016) estimated the cost of the generation and commercial release of one transgenic event by a not-for-profit institution in a single country to be around US \$1.4 million. Although this cost is substantially



*Corresponding author:

E-mail: jjjimenezb@unal.edu.co
 ORCID: 0000-0003-3931-072X

Received: 18 August 2023

Accepted: 23 December 2023

Published: 04 March 2024

¹ Universidad Nacional de Colombia, Ave Cra 30 #45-3, Bogota, Colombia

² Federación Nacional de Cultivadores de Cereales y Leguminosas - FENALCE, Kilómetro 1, vía Cota-Siberia, Vereda El Abra, Cota, Cundinamarca, Colombia

³ Centro de Investigación y Transferencia de Tecnología de la Cadena Agroalimentaria de los Cereales y las Leguminosas - CENICEL, Kilómetro 1, vía Cota-Siberia, Vereda El Abra, Cota, Cundinamarca, Colombia

lower than previously reported, it is still challenging for research institutions or small–medium companies in developing countries, such as Colombia, to procure these budgets.

Another aspect that hinders the commercial release of biotech crops is the complexity of legal aspects, such as intellectual property (IP) rights, institutional agreements, and regulatory framework. For example, the Corporación para Investigaciones Biológicas (CIB) and Universidad Nacional de Colombia developed a GM potato line, which could not be submitted to the commercial authorization process due to problems with material transfer agreements and legal agreements signed by universities and institutions involved in its development (Hincapié and Chaparro-Giraldo 2014).

Patents may also be a difficulty in developing GM crops. However, since 2015, with the expiration of the glyphosate-tolerant Roundup Ready™ soybean patents, the opportunity for a new market for generic GM crops, or agbiogenerics, has been discussed (Jefferson et al. 2015). Two approaches can generate generic GM crops: first, conventional breeding can transfer an event in the public domain to other cultivars of the same species, creating new GM varieties (or hybrids) with the same DNA insert in the same genomic locus as the original GM crop. These are referred to as off-patent events (Rüdelshheim et al. 2018). Alternatively, an entirely new event can be produced by genetically transforming a variety with expression cassettes, genes, or regulatory sequences that have been previously used but are now in the public domain, thus producing an agbiogeneric that does not necessarily have the exact same inserted sequence as prior events and may have a different insertion site in the genome.

To our knowledge, only two off-patent events have been released onto the market, both of them in the United States. The University of Arkansas released two soybean varieties with the GTS 40-3-2 event (Chen 2016, Orazaly et al. 2019), followed by the University of Missouri, which continues to produce new off-patent cultivars with the same event (Chen et al. 2020). The Farmers Business Network has also announced the first generic insect-resistant corn (Bennett 2019); however, at present, we have not been able to find its Biopesticide Registration or FDA approval.

In Colombia, the National Federation of Cereal, Legumes, and Soybean Growers (FENALCE) and the Plant Genetic Engineering Group from Universidad Nacional de Colombia have developed an off-patent corn cultivar by introducing the TC1507 event into an elite Colombian corn line (Jiménez-Barreto et al. 2016). This event confers lepidopteran resistance (Cry1F protein) and glufosinate herbicide tolerance (PAT protein). In 2019, it was granted authorization for commercial cultivation (ICA 2019). Subsequently, national authorities approved its use in feed and food (ICA 2020, INVIMA 2022), becoming the first off-patent event fully released in Latin America.

This note discusses the most relevant aspects that have allowed the commercial release of Fenaltec 22 in Colombia, which may serve as an example of the development of this type of research in other developing countries.

THE PATH TO A GENERIC GM CROP

In addition to crop breeding techniques and molecular analyses, developing an off-patent event involves managing aspects, such as IP rights and complying with the regulatory framework for biosafety. This complexity makes it necessary to analyze these aspects specifically for the country or countries of interest since regulation is specific in each jurisdiction (Parisi et al. 2013).

INTELLECTUAL PROPERTY RIGHTS

Transgenic events are covered by a network of IP rights, mainly represented by patents. Patents are specific to the country or countries in which they are issued. If a patent covering a GM event is not requested in a country and there are no other IP rights protecting it, then the event is in the public domain in that jurisdiction; consequently, it could be used without infringement of IP rights. Notwithstanding, for a genetically modified organism, other aspects such as compliance with the biosafety regulatory framework and legal access to the seeds must be considered.

In terms of IP, the first step is to perform a Freedom to Operate (FTO) analysis to identify the IP rights associated with an event in the country or countries where the event aims to be commercialized. It must cover genetic elements, such as genes, regulatory sequences, expression cassettes, and vectors, and the event itself.

In our case, an FTO analysis for Colombia was conducted for the TC1507 event (Jiménez-Barreto et al. 2016), finding no granted patents or patent applications for the country. An updated patent search for Colombia in the national database (<http://sipi.sic.gov.co/>) was conducted for the period between January 2017 and July 2023, where no new information related to the TC1507 event was found. Internationally, United States patents associated with genes and regulatory sequences have all expired. US patents related to the event itself have expiration dates ranging from 2024 to 2034 (US 7288643 B2, US 7417132 B2, US 7435807 B1, US 7449564 B2, US 7514544 B2, US 7989607 B2, US8901378 B2, US 10023921 B2). These patents claim a DNA molecule that comprises the expression cassette and its flanking regions, a kit for detecting the TC1507 event, a diagnostic method for screening corn seeds, and a method for producing an insect-resistant corn plant by conventional breeding. Some of these patents were also granted in the European Union and countries including Canada, Australia, New Zealand, Brazil, Japan, Taiwan, and South Korea.

Although the oldest transgenic events have no granted patents or requests in Colombia, the number of patent requests related to plant biotechnology has increased in the last few years, probably limiting off-patent use of the most recent events. This could also be the case in other developing countries since first generation transgenic events have been patented in a few countries. Nonetheless, there are differences in the scope of patentability between countries. Thus, it is necessary to conduct a literal examination of patent claims in the jurisdiction of interest to determine the extent of the FTO. For instance, as in other Andean Community countries, Colombia does not allow patenting plants (CAN 2000), unlike the United States. Even though we have found GM crop events patented in Colombia, these patents do not claim seeds, but the GM event is indirectly protected by claiming the DNA molecule and their flanking regions in the host genome (SIC 2023).

Another aspect of consideration is the IP of the genotype, in which the introgression of the transgene is made. In countries that have signed the International Union for the Protection of New Varieties of Plants (UPOV) treaty, such as Colombia, this protection can be achieved through the Plant Breeder's Rights.

If there is no FTO or if it is limited, it would still be possible to use the technology through the negotiation of a license. Nonetheless, performing a full FTO analysis from the beginning of the research project would allow the consideration of viable strategies to avoid wasting time and resources.

Obtaining the GM seeds to be used as a source of the transgene must be done according to national regulations. Transgenic organisms can only be manipulated by companies or institutions that have authorization for developing activities with GMOs. If seeds were to be imported, import authorization would be required. Being cautious about these issues from the beginning of the research is fundamental for an eventual commercial release.

REGULATORY FRAMEWORK

There is a well-established regulatory framework for the biosafety of new GM crops based on the Cartagena Protocol. In short, it is mandatory to perform: i) an environmental risk assessment to obtain authorization for cultivation, ii) risk assessments for feed and food consumption, which require proving that the transgenic plant is as safe as its conventional counterpart through testing their agronomic efficiency, biological efficacy, compositional analysis, and innocuity of the newly introduced proteins (details on the regulatory framework in Colombia are described by Mora-Oberlander et al. 2018). In contrast, generic GM crops are recent, and a pathway for their commercial release has not been clear, as it is dependent on the national regulatory landscape.

One key factor in the development of an off-patent event is maintaining affordable costs for the developer. It is thus convenient to include transgenic events that have already fulfilled biosafety regulatory requirements. Although some information on biosafety may be confidential, there is also public information that can be used to assess biosafety. Today, there is enough public information that supports the biosafety of many transgenic events, especially the oldest ones. Biotech crops have been on the market for more than 26 years, and a lot of information has been produced that supports their safe use. For instance, for the TC1507 event and the Cry and PAT proteins contained in it, we found at least 19 scientific articles that accounted for their biosafety.

Our approach to complying with Colombia's regulatory framework was to supplement our own data with publicly available information. First, we demonstrated that the off-patent event corresponds to TC1507. Molecular characterization

was performed through conventional PCR assays followed by sequencing. The entire expression cassette (6186 bp) was walked through the PCR assays. PCR products were sequenced using the Sanger method. The sequences obtained were analyzed using Chromas V 2.6.6 software and assembled into a final sequence using Bioedit V 7.0.5.3. The sequence obtained was compared with that reported for event GI: GC560390. Four nucleotide changes were found between these sequences. *In silico* translational analysis demonstrated that these changes did not affect the coded proteins. These differences may correspond to errors in the original reported sequence since in the more updated risk assessment of the TC1507 event (EFSA 2017), the applicant amended some nucleotides from the original sequence. Flanking regions were also amplified by PCR assays and sequenced. Overall, 1541 bp upstream and 1006 pb downstream of the event were sequenced. These nucleotide sequences matched those previously reported (Barbour et al. 2008). An enzyme-linked immunosorbent assay (ELISA) confirmed the expression of Cry1F and PAT proteins.

Field trials comparing the agronomic behavior of Fenaltec 22 with conventional and commercial transgenic corn hybrids were conducted in six natural regions in Colombia, each in four different locations. Tests that compared their agronomic efficiency and biological efficacy found that they conserve the major characteristics of the recurrent parental (non-transgenic elite line) and express glufosinate tolerance and resistance to *Spodoptera frugiperda*.

Risk assessment for authorization for animal and human consumption is based on the study of the possible allergenicity or toxicity of the introduced proteins. Cry and PAT proteins have been evaluated on several occasions using the weight-of-evidence approach without any indication that they could be considered allergens (Ladics et al. 2006, Randhawa et al. 2011, Dunn et al. 2017). There is also evidence proving their digestibility, thermolability, and lack of glycosylation (FSANZ 2003, EFSA 2005, Hérouet et al. 2005, US EPA 2005, Schafer et al. 2016).

Oral toxicity tests have been conducted for Cry1F and PAT proteins in mice. No effects related to Cry1F and PAT protein administration were found on body weight, necropsy, or mortality even in high doses (> 5000 mg test material/kg body weight) (FSANZ 2003, EFSA 2005, US EPA 2005).

Subchronic oral toxicity tests have been conducted on Sprague-Dawley rats (MacKenzie et al. 2007) showing no toxicologically significant differences in the variables of nutritional performance, clinical or neurobehavioral signs, ophthalmology, clinical pathology (hematology, clinical chemistry, coagulation, or urinalysis), organ weight, or gross or microscopic pathology outcomes between any pair of treatment groups. Similar results have been obtained when performing subchronic toxicity tests with stacked events containing event TC1507 (Appenzeller et al. 2009).

Although the lack of toxicity and allergenicity of Cry and PAT proteins has been proven, national authorities ask for updated bioinformatics analysis. For Fenaltec 22, Cry1F and PAT protein sequences and all possible open reading frames (ORFs) generated by the TC1507 expression cassette and its flanking regions were compared against the Allergen online V20 allergen database. The search was conducted according to the parameters suggested by the Codex Alimentarius Commission (2009). The comparison indicates that there are no significant matches between the Cry1F and PAT proteins and known allergen sequences. No significant coincidences were found between any of the putative ORFs originating from the *in silico* translation of the expression cassette and their flanking regions in the six reading frames and allergenic substances.

Comparison of the Cry1F and PAT protein sequences, and all possible ORFs generated by the TC1507 expression cassette and its flanking regions was also performed against a curated database of animal toxins and venom proteins (Jungo et al. 2012, UniProt Consortium 2021). No significant matches were found between the Cry1F and PAT proteins and toxin sequences. No significant matches were found between the putative ORFs and toxin sequences.

Nutritional composition equivalence between the TC1507 event and conventional corn has been demonstrated (FSANZ 2003). Additionally, a comparison of nutritional component levels of proximate analytes present in grains and forage tissues was made (Suárez et al. 2022), finding no statistically significant differences between off-patent GM and conventional maize.

Together, these results allow national authorizations for commercial release to be obtained. Fenaltec 22 is in the public domain (with no breeder's protection or associated patents) and is commercialized at the same price as conventional corn seed, in contrast to the higher price of non-generic GM seeds. This was the result of a collaboration between a national private company, Fenalce (which associates corn producers), and a public university, Universidad Nacional de

Colombia, evidencing the importance of this type of joint effort for getting research products to the market. Here, we show a complete path for the commercial release of an off-patent event according to Colombian regulations that can be used as a route for similar developments both in Colombia and other developing countries. Nonetheless, it is necessary to consider the most appropriate approach according to local regulations.

ACKNOWLEDGEMENTS

Our gratitude to the late Dr. Alejandro Chaparro-Giraldo, whose contributions and direction made the development of this research possible. We also thank FENALCE and Fondo Nacional Cerealista for their financial support and contributions.

REFERENCES

- Appenzeller LM, Malley L, MacKenzie SA, Hoban D and Delaney B (2009) Subchronic feeding study with genetically modified stacked trait lepidopteran and coleopteran resistant (DAS-Ø15Ø7-1xDAS-59122-7) maize grain in Sprague-Dawley rats. **Food and Chemical Toxicology** **47**: 1512-1520.
- Barbour E, Bing JW, Cardineau GA, Cressman Jr RF, Gupta M, Hartnett Locke ME, Hondred D, Keaschall JW, Koziel MG, Meyer TE, Moellenbeck D, Narva KE, Nirunskisiri W, Ritchie SW, Rudert ML, Sanders CD, Shao A, Stelman SJ, Stucker DS, Tagliani LA and Van Zante WM (2008) Corn event TC1507 and methods for detection thereof. **US Patent**: US 7417132. Pioneer Hi Bred Int. Du Pont, Dow Agrosciences LLC.
- Bennett C (2019) First-ever generic Bt corn nears farmland. Available at <<http://agweb.com/news/crops/crop-production/first-ever-generic-bt-corn-nears-farmland>>. Accessed on July 10, 2023.
- Brookes G and Barfoot P (2020) Farm income and production impacts from the use of genetically modified (GM) crop technology 1996-2020. **GM Crops Food** **13**: 171-195.
- Brookes G and Barfoot P (2022) Environmental impacts of genetically modified (GM) crop use 1996–2018: impacts on pesticide use and carbon emissions. **GM Crops Food** **11**: 215-241.
- CAN - Comisión de la Comunidad Andina (2000) **Decisión 486**. Régimen común sobre propiedad industrial. Lima.
- Chen P (2016) Soybean cultivar UA 5414RR. **US patent**: US 9326478 B2. Board of Trustees of the University of Arkansas.
- Chen P, Shannon G, Ali M, Scaboo A, Smothers S, Clubb M, Selves S, Vieira CC, Mitchum MG, Nguyen HT, Li Z, Bond J, Meinhardt C, Usovsky M, Li S, Mengistu A and Robbins RT (2020) Registration of ‘S14-9017GT’ soybean cultivar with high yield, resistance to multiple diseases, and high seed oil content. **Journal of Plant Registrations** **14**: 347-356.
- Codex Alimentarius Commission (2009) **Codex alimentarius. Foods derived from modern biotechnology**. FAO/WHO, Rome, 85p.
- Dunn SE, Vicini JL, Glenn KC, Fleischer DM and Greenhawt MJ (2017) The allergenicity of genetically modified foods from genetically engineered crops: A narrative and systematic review. **Annals of Allergy, Asthma & Immunology** **119**: 214-222.
- EFSA - European Food Safety Authority (2005) Opinion of the scientific panel on genetically modified organisms [GMO] on an application (reference EFSA-GMO-NL-2004-02) for the placing on the market of insect-tolerant genetically modified maize 1507, for food use, under Regulation (EC) No 1829/2003 from Pioneer Hi-Bred International/ Mycogen Seeds. **EFSA Journal** **182**: 1-22.
- EFSA - European Food Safety Authority (2017) Scientific opinion on an application for renewal of authorisation for continued marketing of maize 1507 and derived food and feed submitted under Articles 11 and 23 of Regulation (EC) No 1829/2003 by Pioneer Overseas Corporation and Dow AgroSciences LLC. **EFSA Journal** **15**: 4659.
- FSANZ - Food Standards Australia New Zealand (2003) Final assessment report application a446 insect-protected and glufosinate ammonium-tolerant corn line 1507. Available at <http://www.fao.org/fileadmin/user_upload/gmfp/docs/ACF18.pdf>. Accessed on July 30, 2023.
- Hérouet C, Esdaile DJ, Mallyon BA, Debruyne E, Schulz A, Currier T, Hendrickx K, Van der Klis RJ and Rouan D (2005) Safety evaluation of the phosphinothricin acetyltransferase proteins encoded by the pat and bar sequences that confer tolerance to glufosinate-ammonium herbicide in transgenic plants. **Regulatory Toxicology and Pharmacology** **41**: 134-149.
- Hincapié VP and Chaparro-Giraldo A (2014) Estudio de la libertad de operación para una línea genéticamente modificada de papa (*Solanum tuberosum* L.). **Revista Colombiana de Biotecnología** **16**: 119-128.
- ICA - Instituto Colombiano Agropecuario (2019) **Resolución 13025 de 26 de agosto de 2019**. “Por la cual se autoriza a la Federación Nacional de cultivadores de cereales, leguminosas y soya -FENALCE, siembras comerciales de los genotipos de maíz que contengan el evento TC1057, en las subregiones naturales Caribe Húmedo, Caribe Seco, Valle Geográfico del Río Cauca, Valle Geográfico del Río Magdalena, Orinoquía y Área Cafetera”. Bogotá.
- ICA - Instituto Colombiano Agropecuario (2020) **Resolución 82354 de 29 de diciembre de 2020**. “Por la cual se autoriza a la Federación Nacional de cultivadores de cereales, leguminosas y soya - FENALCE, el uso de maíz (*Zea mays*) evento TC1507 para consumo directo y/o como materia prima para la elaboración de alimentos para animales domésticos”. Bogotá.
- INVIMA - Instituto Nacional de vigilancia de Medicamentos y Alimentos (2022) **Resolución 2022500207 del 15 de febrero de 2022**. “Por la cual se autoriza el uso de maíz Fenaltec 22 como alimento o materia prima para la elaboración de alimentos para consumo humano. Bogotá.

- Jefferson DJ, Graff DG, Chi-Ham CL and Bennett AB (2015) The emergence of agbiogenetics. **Nature Biotechnology** **33**: 819-823.
- Jiménez-Barreto J, Chaparro-Giraldo A, Mora-Oberlaender J and Vargas-Sánchez JE (2016) Molecular characterization and freedom to operate analysis of maize hybrids from genetically modified and Colombian varieties. **Agronomía Colombiana** **34**: 309-316.
- Jungo F, Bougueleret L, Xenarios I and Poux S (2012) The uniprotkb/swiss-prot tox-prot program: A central hub of integrated venom protein data. **Toxicon** **60**: 551-557.
- Ladics GS, Bardina L, Cressman RF, Mattsson JL and Sampson HA (2006) Lack of cross-reactivity between the *Bacillus thuringiensis* derived protein Cry1F in maize grain and dust mite Der p7 protein with human sera positive for Der p7-IgE. **Regulatory Toxicology and Pharmacology** **44**: 136-143.
- MacKenzie SA, Lamb I, Schmidt J, Deege L, Morrissey MJ, Harper M, Layton RJ, Prochaska LM, Sanders C, Locke M, Mattsson JL, Fuentes A and Delaney B (2007) Thirteen week feeding study with transgenic maize grain containing event DAS-Ø15Ø7-1 in Sprague-Dawley rats. **Food and Chemical Toxicology** **45**: 551-562.
- McDougall P (2011) The cost and time involved in the discovery, development and authorization of a new plant biotechnology derived trait. **Consultancy Study for Crop Life International**. Midlothian, 24p.
- Mora-Oberlaender J, Castaño-Hernández A, López-Pazos SA and Chaparro-Giraldo A (2018) Genetic engineering of crop plants: Colombia as a case study. In Kuntz M (ed) **Transgenic plants and beyond**. Advances in Botanical Research, vol 86. Academic Press, London, p. 169-206.
- Orazaly M, Florez-Palacios L, Manjarrez-Sandoval P, Mozzoni L, Dombek D, Wu C and Chen P (2019) Registration of 'UA 5715GT' soybean cultivar. **Journal of Plant Registrations** **13**: 31-37.
- Parisi C, Rodríguez-Cerezo E and Thangaraj H (2013) Analysing patent landscapes in plant biotechnology and new plant breeding techniques. **Transgenic Research** **22**: 15-29.
- Randhawa GJ, Singh M and Grover M (2011) Bioinformatic analysis for allergenicity assessment of *Bacillus thuringiensis* Cry proteins expressed in insect-resistant food crops. **Food and Chemical Toxicology** **49**: 356-362.
- Rüdelshaim P, Dumont P, Freyssinet G, Pertry I and Heijde M (2018) Off-patent transgenic events: challenges and opportunities for new actors and markets in agriculture. **Frontiers in Bioengineering and Biotechnology** **6**: 71.
- Schafer BW, Embrey SK and Herman RA (2016) Rapid simulated gastric fluid digestion of in-seed/grain proteins expressed in genetically engineered crops. **Regulatory Toxicology and Pharmacology** **81**: 106-112.
- Schiek B, Hareau G, Baguma Y, Medakker A, Douches D, Shotkoski F and Ghislain M (2016) Demystification of GM crop costs: Releasing late blight resistant potato varieties as public goods in developing countries. **International Journal of Biotechnology** **14**: 112-131.
- SIC – Superintendencia de Industria y Comercio (2023) **Resolución 29228 de 30 de mayo de 2023**. Por la cual se otorga una patente de invención titulada: Evento Élite EE-GM5 y métodos y kits para identificar dicho evento en muestras biológicas. Bogotá.
- Suárez HD, Benitez DA, Chaparro-Giraldo A and Acosta A (2022) Equivalence of grain and forage composition in corn hybrid (*Zea mays* L.) from genetically modified off-patent (event TC1507) and non-genetically modified conventional corn. **Agronomía Colombiana** **40**: 155-164.
- UniProt Consortium (2021) UniProt: the universal protein knowledgebase in 2021. **Nucleic Acids Research** **49**: D480-D489.
- US EPA - United States Environmental Protection Agency (2005) Biopesticides registration action document – *Bacillus thuringiensis* Cry1F corn. Available at <https://archive.epa.gov/pesticides/biopesticides/web/pdf/brad_006481.pdf>. Accessed on July 30, 2023.