

Global Trends and Developments in Confined Field Trials with Genetically Modified Plants

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Abstract

Confined field trials (CFTs) are a key component in the research and development process for genetically modified (GM) plants. They are also an integral part of the step-by-step regulatory approach and consistent with the precautionary approach. By monitoring the annual number of CFTs and the species and traits of the GM plants involved, one can gain insight into future trends. However, there are several challenges to creating a simple inventory. This article reports a systematic way to construct and analyse such an inventory. Despite a decrease in the annual number of CFTs in recent years, a huge number of CFT applications are still submitted annually (on average, 10,000 CFTs) in a wide range of countries across all continents. Although major commodity crops are the focus of most CFTs, a variety of minor crops are tested on a much lower scale. This may indicate that fewer CFTs of minor crops and/or for adaptations to local markets are required for market introduction. In addition, for crops with a longer generation time (such as trees), applications for subsequent CFTs are likely to be less frequent. Although pioneering biotechnology traits (such as herbicide tolerance and insect resistance) remain the most dominant traits, resistance to other pest and diseases, as well as abiotic stress tolerance, are also being investigated. Our inventory currently holds records of nearly 161,000 CFTs undertaken since 2001. This broad experience should provide an ample basis for regulatory systems to streamline their processes so that minor and more local crops with financially less attractive traits can also progress safely to market introduction.

Keywords: CFTs, confined field trials, databases, global trends, GM plants, inventory.

Riassunto

Le prove in campo confinato (CFT) sono una componente chiave del processo di ricerca e sviluppo delle piante geneticamente modificate (GM). Sono anche parte integrante dell'approccio normativo graduale in linea con il principio precauzionale. Monitorando il numero annuale di CFT, le specie e le caratteristiche delle piante GM coinvolte, si può avere una prospettiva sulle tendenze future. Tuttavia diverse sfide ostacolano la creazione di un inventario semplice. In questo articolo si indica un modo sistematico di costruire e analizzare questo tipo di inventario. Nonostante una diminuzione del numero di CFT negli ultimi anni, un numero enorme di applicazioni viene ancora presentato annualmente (in media 10.000) in una vasta gamma di paesi in tutti i continenti. Sebbene le principali colture di materie prime rappresentino il focus della maggior parte dei CFT, una varietà di colture minori viene testata su scala molto inferiore. Questo potrebbe indicare che sono necessari meno CFT di colture minori e/o per l'adattamento ai mercati locali per realizzare l'introduzione sul mercato. Inoltre, per le colture con un tempo di crescita più elevato (come gli alberi), è probabile che le applicazioni per i CFT avvengano con minore frequenza. Sebbene le caratteristiche pionieristiche della biotecnologia, come la tolleranza agli erbicidi e la resistenza agli insetti, rimangano i tratti più dominanti, sono state studiate anche altre resistenze ai parassiti e alle malattie, nonché le tolleranze agli stress abiotici. Il nostro inventario attualmente conta circa 161.000 CFT dal 2001. Questa vasta esperienza dovrebbe fornire un'ampia base affidabile per i sistemi normativi per snellire i loro processi in modo che anche le colture minori e locali meno attenti dal punto di vista economico possano progredire in sicurezza verso l'introduzione sul mercato.

1. INTRODUCTION

In general, the development of genetically modified (GM) crops follows a standard trajectory of research, development and marketing stages (Rüdelsheim, 2015). In a preparatory phase, basic research might be conducted in a model species. In a subsequent phase, a proof-of-concept study is undertaken to demonstrate delivery of the planned strategy, i.e. expression of the intended trait in a crop of economic relevance. This can be the trigger to embark on a full-scale development project aimed at the commercial introduction of an elite event into locally adapted germplasm.

Most early phase activities are performed in laboratories, growth rooms and glasshouses. These are followed by small-scale, proof-of-concept field trials and then by larger trials to further characterise and multiply material (principally seed) of the transformation events. The trials take place in different geographical areas and different growing seasons to test performance under diverse growing conditions. Each step involves rigorous selection of a single elite event that may be ready for market introduction.

Confined field trials (CFTs; i.e. field trials with some form of regulatory restriction and requirement to confine the GM material) are therefore seen as an indicator of particular scientific interest. On the other hand, repeated CFTs with an increasing number of trials and acreage typically indicates a development programme intended for commercial/large-scale market introduction. Although information on the actual performance of CFTs is not systematically available, some information about regulatory applications for CFTs is publicly available in most countries with genetically modified organism (GMO) legislation. An inventory of CFT applications can thus indicate which trait/crop combinations to expect for commercial release in the next 5–10 years.

Following Organisation for Economic Co-Operation and Development (OECD) surveys (van Beuzekom & Arundel, 2006; 2009) analysing trends in biotechnology developments, more specifically in GM crops, we previously conducted a survey of CFTs for the Netherlands Commission on Genetic Modification (COGEM) for the period 2009–2013 (Rüdelsheim & Smets, 2014). This review provides an update for the 2014–2017 period and analyses trends or trend changes compared with previous reports.

2. METHODOLOGY

2.1. Scope of the Survey

The geographical scope of this updated survey was set to cover all OECD Member States and/or Parties to the Cartagena Protocol on Biosafety (CPB). Argentina, although neither an OECD member nor a Party of the CPB, was included in the previous survey because it is an important country in terms of the number of CFTs undertaken. Since then, however, data on CFTs for Argentina are no longer publicly

available. In addition, Brazil ceased updating the national authority's website in 2014; therefore, the information found there is unreliable.

This survey covers all CFTs in the period from 01 January 2014 to 31 December 2017, inclusive. Public databases and other information sources were also searched for CFT applications that were submitted and/or approved before this period but intended to be conducted in the survey period.

Limiting the scope of the survey to CFTs automatically imposes a focus on trials with regulated GMOs. Whenever and wherever a GM plant is approved for commercial introduction, field use would no longer be considered a CFT. Yet, as commercial approval may not be obtained simultaneously in every country, it may still be reported as a regulated CFT in some locations. Similarly, as commercially approved GM plants may be used as comparators in the evaluation of other regulated GM plants, they will continue to be reported.

The survey covers GM higher plants, including arable crops, vegetables, non-agronomic higher plants, shrubs and trees. Care was taken to include applications for scientific interest, for food and feed, and for industrial purposes (e.g. energy crops, pharmaceuticals). Algae, mosses or organisms other than higher plants were excluded.

2.2. Information Gathering

2.2.1. Sources of information

A search was made for information on CFTs worldwide by adopting a stepwise approach:

1. consultation of databases established by relevant competent authorities;
2. review of information provided to the public;
3. analysis of decision documents of advisory committees and/or authorities;
and
4. verification of communications through the Biosafety Clearing House hosted by the Secretariat of the Convention on Biological Diversity.

In cases where this approach did not reveal any information on a possible CFT, additional literature and publications were searched. Some examples are the attaché reports on biotechnology of the United States of America's Department of Agriculture (USDA) Foreign Agriculture Service¹, communications via Seedquest² and the Crop Biotech Update service of the International Service for the Acquisition of Agri-Biotech Application (ISAAA)³. This additional effort did not guarantee a complete overview because it is influenced by the ability to trace initiatives; however, it was deemed

¹<https://gain.fas.usda.gov/>

²<https://www.seedquest.com/portal/biotechnology/news>

³<http://www.isaaa.org/kc/cropbiotechupdate/>

necessary to ensure that information would also be included from countries that have not yet set up mechanisms to routinely provide information on CFTs.

2.2.2. Processing information

Authorities use different base units for approving and reporting CFTs of GM plants (see Section 3.5.). Consequently, the methodology selected to integrate these data into a single dataset can significantly influence the resulting analyses. The dataset structure of Rüdelsheim & Smets (2014) was selected for use in the present study (Table 1).

Table 1. Information structure of the dataset for this review

Dataset topic	Information	Specification
Reference number	<ul style="list-style-type: none"> • Specific internal reference number • Official reference number from authority 	Reference numbers used to identify specific applications. As an authority reference number can refer to a number of CFTs during the authorised period (see later), an internal reference was also included
Applicant	<ul style="list-style-type: none"> • Applicant name • Applicant type 	Type may be not-for-profit (public) research organisations or industry
Developer	<ul style="list-style-type: none"> • Developer name • Developer type 	Type may be not-for-profit (public) research organisations or industry
Approving authority	<ul style="list-style-type: none"> • The national agency/office that approves CFT applications 	–
Application year	<ul style="list-style-type: none"> • The year that the CFT application was submitted 	–
Approval year	<ul style="list-style-type: none"> • The year that the CFT application was approved 	–
Validity period	<ul style="list-style-type: none"> • The period for which the approval/permit is valid 	Expressed in months
Information source	<ul style="list-style-type: none"> • The source from which the data were retrieved 	–
High-level geography	<ul style="list-style-type: none"> • Regional group to which the country belongs 	This can be Europe, North America, Latin America, Africa, Asia, Australia or New Zealand
Trial country	<ul style="list-style-type: none"> • Country in which the CFT was authorised to take place 	–

Dataset topic	Information	Specification
Trial location in country	<ul style="list-style-type: none"> Location within the country 	This can be a province, state or agronomic region
Trial site number	<ul style="list-style-type: none"> The number of trials within a location 	–
Trial year	<ul style="list-style-type: none"> Year in which the trial began 	–
Trial period	<ul style="list-style-type: none"> The duration of the growing season 	Expressed in months
Trial area	<ul style="list-style-type: none"> The dimensions of the trial 	When more than one trial per location, an average trial area is included
Trial number	<ul style="list-style-type: none"> Amount of plants/seeds 	The amount of material, e.g. the number of trees or the weight of seeds
Receptor	<ul style="list-style-type: none"> The scientific name of the GM plant species The common name of the GM plant species 	–
Aggregated class feature	<ul style="list-style-type: none"> Indication of all trait classes and types 	See subsequent section on trait classification
Individual traits	<ul style="list-style-type: none"> Trait Gene 	The specified trait type

2.2.3. Trait classification

The classification system was a refinement of the version used by USDA APHIS (2011a) with respect to grouping types of traits. A tiered structure was established (Table 2); examples of all trait classes are shown in Table 3.

Table 2. Tiered trait classification used in this review

Level	Description	Example
Trait class	All-encompassing generic classification of the trait	Agronomic properties
Trait type	General description of the trait	Herbicide tolerance
Trait	Specific description of the trait or phenotype	Glyphosate tolerance
Function	Where possible, details of the gene or mode of action were included	<i>CP4 epsps</i>

Table 3. Trait categories used in this study

Trait class	Examples of trait type	Examples of traits/phenotypes
Agronomic properties	Abiotic stress tolerance	<ul style="list-style-type: none"> • Drought tolerance • Cold tolerance • Tolerance to specific environmental stresses • Nitrogen use efficiency
	Plant biology	<ul style="list-style-type: none"> • Male sterility • Yield increase • Altered growth rate
	Herbicide tolerance	<ul style="list-style-type: none"> • Glyphosate tolerance • Dicamba tolerance
Biotic stress resistance	Bacteria resistance	<ul style="list-style-type: none"> • Bacterial leaf blight resistance
	Fungus resistance	<ul style="list-style-type: none"> • <i>Sclerotinia</i> resistance • <i>Botrytis cinerea</i> resistance
	Insect resistance	<ul style="list-style-type: none"> • Lepidoptera resistance • Coleoptera resistance
	Nematode resistance	<ul style="list-style-type: none"> • Soya bean cyst nematode resistance
	Virus resistance	<ul style="list-style-type: none"> • Cucumber mosaic virus resistance • Potato virus Y resistance
Product specifications	Product quality	<ul style="list-style-type: none"> • Delayed fruit ripening • Altered amino acid profile • Modified seed storage proteins • Enhanced floral characteristics • Increased solids in fruits
	Product systems	<ul style="list-style-type: none"> • Pharmaceutical protein production
Other traits	Breeding aids	<ul style="list-style-type: none"> • Recombinase gene
	Marker genes	<ul style="list-style-type: none"> • Screenable marker • Selectable marker
	Other	<ul style="list-style-type: none"> • Genetic studies

2.3. Analysis

Data were retrieved from the databases described above. Regulatory documents were also searched for as much data as possible; the resultant data were entered, with corrections when necessary, into the dataset.

All calculations used the “CFT” as the base unit. Calculations were made per country in which the trials were conducted. In addition, the distribution of trait types was analysed per crop and per trial. This was calculated as an aggregate trait type (as opposed to an individual phenotype) since one gene may result in one or more trait specifications. For example, for a gene conferring both drought tolerance and salt tolerance, the trait was recorded only once, as abiotic stress tolerance. Likewise, calculations for determining the distribution of companies versus public research institutions were based on the number of trials instead of the number of individual traits.

3. METHODOLOGICAL CHALLENGES

Before discussing the results of the survey, it is necessary to report the various methodological challenges encountered and how they were addressed because this will certainly have influenced the interpretation of results.

3.1. Not All Countries Systematically Report on CFTs of GM Crops

Some authorities make information on CFTs publicly available. For most of these, this is part of their national legal framework and may, for example, be integrated into a public consultation process. On the other hand, many countries do not (yet) share information related to processing CFT applications with the public; as a consequence, information had to be sought more indirectly. In such cases, the extracted information is merely indicative in nature.

Of course, countries that have not yet received or processed CFT applications for GM plants have no information to share. In such cases, the main issue was to confirm that the lack of information reflected an absence of CFTs.

3.2. Extracting Information that is Presented in Different Forms

When performing this survey, information had to be extracted from different types of documents to obtain the data needed for the analysis. Information can be presented in very different forms, for example by different authorities.

- *In databases and official lists.* These may be issued by the competent authorities themselves or commissioned by the authorities. Data may be presented as simple lists with one record per trial or event, as is the case in Japan.
- *In documents submitted by applicants.* These documents contain details of the GMO and the envisaged trial, including a summary of the environmental risk assessment, e.g. as in the European Union (EU) or Australia.
- *In decision documents prepared by the competent authority.* In Latin America (e.g. Colombia and Uruguay), information often takes the form of resolutions (*Resoluciones*) amidst other decisions taken by the authorities.

Even when official lists were available, the extracted data required careful verification because not all entries automatically matched the review dataset design. It was more difficult if documents were only available in a language not readily understood by the authors. In such cases, translation tools were used.

3.3. Dealing with Data Gaps

The available data ranged from very detailed descriptions of plants, genes and trial location to as little information as the plant and location with no indication of the trait (e.g. in Chile). Table 4 gives a comparison of the data available in different countries.

Table 4. Overview of information available in public databases

Country	Plant species	Applicant	Site	Area	Application year	Trial year	Gene	Promoter/terminator	Trait	Application/permit	Event name	OECD unique identifier
Australia	√	√	√	√	√	√	√	√	√	√	±	–
Canada	√	√	√	–	–	√	–	–	√	–	–	–
Chile	√	√	√	√	–	√	–	–	–	–	–	±
Colombia	√	√	√	–	–	–	√	√	√	√	–	√
EU	√	√	√	√	√	√	√	√	√	√	±	±
India	√	√	–	–	–	√	√	–	√	–	√	√
Japan	√	√	√	–	–	√	–	–	√	–	√	√
Kenya	√	√	–	–	–	√	–	–	√	–	–	±
Mexico	√	√	√	√	√	√	–	–	√	√	–	√
Philippines	√	√	–	–	√	√	–	–	√	√	–	–
South Africa	√	√	–	*	–	√	–	–	√	–	√	√
Uruguay	√	√	–	–	–	√	–	–	–	–	–	√
USA	√	√	√	±	√	√	±	±	√	√	–	–

* Number of seed. √: available; ±: sometimes available; –: unavailable.

Some key differences are that:

- the trial site location may be reported with a fine level of detail, such as an individual village (e.g. EU) or GPS coordinates (e.g. Chile) or instead with only a generic indication of the region or state (e.g. Canada, the USA);

- the trial dimensions are not always indicated; even when indicated it is often unclear whether, for example, border rows were included or excluded. South Africa only records the amount of seed that is permitted;
- most countries list submitted CFT applications while a few only list CFTs that were actually performed (e.g. Canada, Chile). Consequently, the study may overestimate the number of CFTs in countries that only list applications or approvals (an approval is no guarantee that the trial will actually be performed);
- information on the genes and associated DNA regulatory sequences is often not disclosed because of confidentiality issues – often the case for developers with commercial intent. Public researchers usually list all of the genes involved, either with (e.g. Australia, EU) or without the associated DNA regulatory sequences (e.g. USA). Information can sometimes be acquired indirectly if not indicated in the public databases. Indeed, some of the trial material may already be approved for commercialisation or derived from such events, either locally or in other countries, and will therefore have an assigned OECD unique event identification code. That code enables searching for detailed information on the introduced genetic material. An example of a database for commercial events is the ISAAA GM Approval Database⁴. Traits are listed or can be derived from the description of the GM plant. Canada only provides a general trait class without further specification;
- although every permit will have some identification code, this code is not always presented; and
- event names or unique identifiers are not always available.

Information gaps were addressed in the review as follows:

- when no number of sites was provided, a single CFT (value = 1) was assigned;
- when the trial year was unknown, the year of approval or, in some cases, the application year was assigned;
- any absence of information was noted; and
- CFTs for which traits were unspecified were not included in trait distribution calculations.

3.4. Discrepancies in GMO Definition

A GMO is generally defined in the national legislation of each country. Differences in legal definitions ultimately lead to competent authorities adopting different regulatory positions on whether a CFT permit or notification for specific plant types is required. This is illustrated in the following comparison of three fundamentally different approaches: in the EU, the USA and Canada. As it was impossible for the authors to trace all of the details in the databases, all information in the applicable legislation was included.

⁴<http://www.isaaa.org/gmapprovaldatabase/>

3.4.1. In the EU

In Europe, Directive 2001/18 (EC, 2001) defines a GMO as:

“an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination.”

Included techniques (i.e. involved in creating a GMO) are listed in Annex I A, Part 1:

1. *recombinant nucleic acid techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation;*
2. *techniques involving the direct introduction into an organism of heritable material prepared outside the organism including micro-injection, macro-injection and micro-encapsulation;*
3. *cell fusion (including protoplast fusion) or hybridisation techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally.*

Excluded techniques concerning the following processes appear in Annex I A, Part 2:

1. *in vitro fertilisation;*
2. *natural processes such as: conjugation, transduction, transformation;*
3. *polyploidy induction.*

Further excluded techniques/methods are listed in Annex I B:

1. *mutagenesis;*
2. *cell fusion (including protoplast fusion) of plant cells of organisms which can exchange genetic material through traditional breeding methods.*

In the EU, stacked events (i.e. genetic modifications that are combined through conventional breeding) are considered “new” GMOs. Even if the parent GMOs have each been approved, CFTs of stacked events require a permit and were therefore included in the study.

Another recent consideration is how so-called new breeding techniques are or will be considered by regulatory authorities. In 2008, the European Commission established a working group to evaluate a set of new techniques used in plant breeding. The goal was to clarify whether products obtained via these techniques are subject to the prevailing GMO legislation. So far, this evaluation has not been completed. However, CFTs of the products of cisgenesis (e.g. B/NL/15/L01, a cisgenic apple with elevated

anthocyanin levels) have been subject to GMO legislation, although some have argued that cisgenic products should not be considered GMOs.

3.4.2. In the USA

In the USA, as outlined in Title 7 § 340.1 of the Code of Federal Regulations (USDA APHIS, 2011b), a plant is regulated on the basis of potentially being a regulated article, as related to a plant pest:

Regulated article. Any organism which has been altered or produced through genetic engineering, if the donor organism, recipient organism, or vector or vector agent belongs to any genera or taxa designated in 340.2 and meets the definition of plant pest, or is an unclassified organism and/or an organism whose classification is unknown, or any product which contains such an organism, or any other organism or product altered or produced through genetic engineering which the Administrator determines is a plant pest or has reason to believe is a plant pest. Excluded are recipient microorganisms which are not plant pests and which have resulted from the addition of genetic material from a donor organism where the material is well characterized and contains only non-coding regulatory regions).

Genetic engineering is defined (USDA APHIS, 2011b) as:

“the genetic modification of organisms by recombinant DNA techniques.”

In the USA, stacked events bred from deregulated transformation events do not need to go through the regulatory process again, provided that each of the progenitor components has been positively assessed for commercial cultivation and that their breeding does not raise any new concerns.

For products of new breeding techniques, the USDA issues opinions on a case-by-case basis (USDA APHIS, 2018) as part of their mandate under their Plant Protection Act.

3.4.3. In Canada

In Canada, all plants with novel traits (PNTs) are regulated, regardless of which breeding technique was used in their production (CFIA, 1994):

[A] Plant with Novel Traits (PNT) is a plant that contains a trait which is both new to the Canadian environment and has the potential to affect the specific use and safety of the plant with respect to the environment and human health. These traits can be introduced using biotechnology, mutagenesis, or conventional breeding techniques.

Directive 94–08 (CFIA, 1994) states that:

A new variety of a species is subject to the notification and authorization requirements of the Seeds Regulations when it possesses trait(s) novel to that species in Canada, i.e.,

1. *the new trait is not present in stable, cultivated populations of the plant species in Canada, or*
2. *the trait in the plant species is present at a level significantly outside the range of that trait in stable, cultivated populations of that plant species in Canada.*

Canada therefore has a product-based regulatory system for PNTs. It is the presence of a novel trait in a plant, irrespective of the method used to introduce it, which triggers the notification and authorisation requirements under the Seeds Regulations. PNTs may be developed through mutagenesis, somaclonal variation, intra- and inter-specific crosses, protoplast fusion, recombinant DNA technology or other techniques. The consequence for this study is that field trials with GM plants (i.e. CFTs) cannot be distinguished from trials with non-GM plants. Therefore, the number of trials reported for Canada might overestimate the actual amount of trials with GMOs.

Further, for scientific research purposes or technical data gathering, CFTs do not require a notification for stacked events (i.e. plant lines developed by conventional crossing of two or more authorised PNTs) provided that the trial size is less than regular CFT size restrictions, as specified in Section 3.2 of Directive Dir2000–07 (CFIA, 2000).

3.5. Determining the Basic Units of CFTs

When performing a comparative analysis, the units being compared must be clearly defined. However, authorities regulate CFTs in different ways, with some allowing groups of similar CFTs to be included in a single application or permit; if not corrected for, this can be important source of error. The decisions made in defining CFTs are listed below.

- Some countries require one application per trial, regardless of the number of events or gene constructs that are tested within that trial (e.g. USA), whereas others require one notification per gene construct (e.g. Japan, Romania, Spain, Sweden). In the latter case, an application is needed for each gene construct used, when a CFT is designed to test the combined effects of the constructs. For the purposes of this study, a CFT was defined as a single location where specific events are tested together in a certain year. For practical reasons, no distinction was made between events or gene constructs because this cannot always be ascertained from the databases. The USDA APHIS database⁵, for example, mentions traits and trait specifications, sometimes even genes, but it is unclear whether they are present in one event or in several or in a combination. Cases in which several gene constructs with separate permits were grown together in one trial were counted as one CFT.

⁵https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permits-notifications-petitions/sa_permits/ct_status

- No distinction was made between, for example, efficacy trials and trials for regulatory purposes, if conducted at the same location. They were counted as one CFT.
- When only the trial region or state was reported, the possibility that more than one location was chosen within the region or state could not be excluded. In this case, only one trial was counted, most probably resulting in underestimation of the number of field trials (e.g. Colombia). Cases in which multi-location trials were involved but the exact number was not indicated were counted as two CFTs, again probably leading to an underestimation (e.g. India). Apart from a few exceptions, the exact location (municipality) was reported for EU Member States. Consequently, counting the locations gave an accurate number of trials. In databases of the Philippine, South African and Uruguayan authorities, no location is provided. For these countries, every field trial application/permit was therefore counted as an individual CFT. However, this approach will certainly lead to an underestimation. The Mexican authority lists the state but only sometimes the municipality, thus skewing the calculation. For Chile, every field trial is described by its coordinates; therefore, the exact number of trial sites was known for this country only. This approach resulted in an over-representation of Chilean trials compared with other countries in Latin America and worldwide.
- The trial year was noted as indicated in the application or permit, if available. If only the year of the application submission was known, then this year was used in the study, while acknowledging that this might cause a difference of one year compared with the actual trial year. For trials conducted in the southern hemisphere, around the equator and even in Puerto Rico and Hawaii (with counter-season applications) where the annual crops span two calendar years, only the year of sowing or planting was considered. Further, for perennial crops such as trees and some forage crops, the year of planting was taken as the reference year. Winter-grown crops (for example, wheat [*Triticum aestivum*]) were treated similarly. For sugarcane and banana, a ratoon crop was not counted as a new trial; only when the derived cuttings were planted were they considered as a new trial.
- Although it is possible for some crops to complete two to three generations within a single year, evidence for this was not apparent from the databases; therefore, this case was still counted as one trial.
- Trait classes and types were inventoried per trial. As a trait type may include different compounds within a trial, this may result in underestimation. This case was mostly crop-specific and therefore analysed per plant species.
- Most countries list CFT notifications, permit applications or issued permits. However, the fact that these processes were completed does not mean that the trial actually took place. Only Canada and Chile list the performed CFTs. This discrepancy, however, did not interfere with the purpose of this study as all research and development (R&D) intentions were included.

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- For the comparison between research institutes and companies, the CFT was taken as the basic unit.
 - Traits were counted as aggregated trait types per CFT. This meant that for a single trial, the “plant biology” trait for example, could only be included only once because it was not always clear how many different trait/gene combinations for this trait type were being tested.

3.6. Conclusion

While the review methodology should ideally be straightforward, different challenges required adjustments related to dataset collection and interpretation. These adjustments might have led to either an over- or underestimation; however, it was concluded that they would not significantly influence the overall outcome of the study. Nevertheless, they have been described for future reference and to develop alternative approaches.

4. RESULTS FOR 2014–2017

4.1. Number of CFTs Worldwide

The number of CFTs per year worldwide gradually decreased during the review period (Fig. 1), especially in North America (Canada and the USA). Worldwide, the numbers decreased from 14,307 in 2014 to 6346 in 2017. The numbers for the USA also fell from 10,442 in 2014, to 3768 in 2017. The number of trials in Canada also decreased, even though they may include CFTs for non-GMO crops (as explained above).

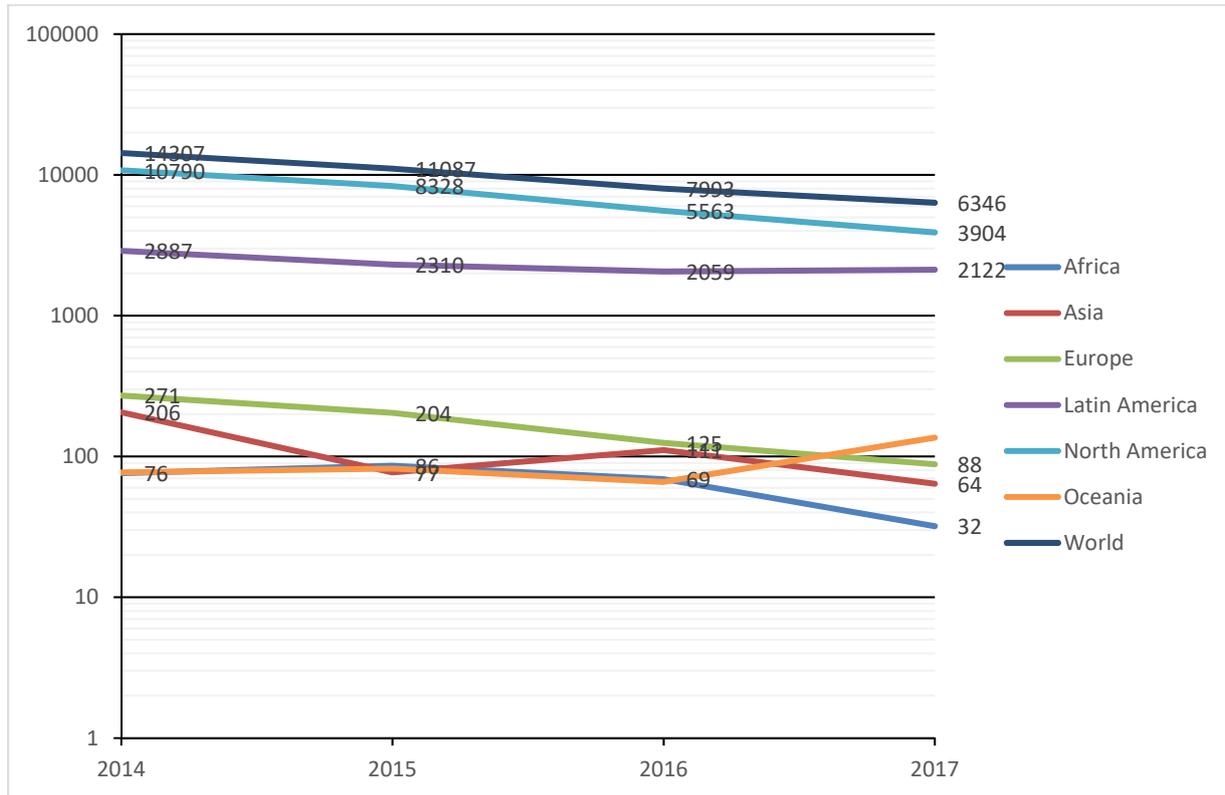


Figure 1. Total number of field trials per year worldwide, 2014–2017

In Europe, the number of CFTs continued to decrease. Most trial applications were received in Sweden, followed by Romania and the Netherlands. However, the actual number of trials conducted was much lower, as applications are often valid for up to 5 years, and sometimes even to 10 years. Therefore, any permits obtained prior to 2014 remained valid during the review period but, again, there was no indication of whether each CFT was actually performed. A general trend in the EU in recent years has been a drastic reduction in the number of submitted applications.

In Africa, the number of CFTs remains low, although more countries are gradually implementing the necessary regulatory infrastructure to enable them to process applications. Almost 66% of all African CFTs were conducted in South Africa, although a general decrease was also noted there. Other prominent countries (with more than 10 CFTs in the study period) were Burkina Faso, Ghana, Kenya, Malawi and Nigeria.

In Asia, China is the biggest player in GM crop development. However, since relatively little data are available, the total number of CFTs for this continent remains low. Except for in India, the exact numbers of field trials are not reported. Moreover, for some other Asian countries, data was obtained indirectly, i.e. not from the regulatory authority websites; as a consequence, conservative estimates were made. In Latin America, the most important countries in terms of CFTs, i.e. Argentina and Brazil, were not included because they no longer make this data publicly available. Based on the available data, the biggest player in the region is Chile followed by Mexico. In this region, field trials

are performed for regulatory purposes for entry onto the domestic market and as a counter-season opportunity for companies based in the northern hemisphere.

4.2. Crops

Of all the GM crops that underwent field testing (Fig. 2), maize (*Zea mays*) was dominant, followed by soya bean (*Glycine max*), cotton (*Gossypium hirsutum*) and oilseed rape (*Brassica napus*). Fewer trials involved other commodity crops, such as potato (*Solanum tuberosum*), rice (*Oryza sativa*), sugar beet (*Beta vulgaris*) and wheat.

Most maize and soya bean CFTs took place in the USA, while Mexico took the lead for cotton. Oilseed rape CFTs were predominant in Chile, where counter-season seed multiplication trials were performed. Interestingly, the number of wheat CFTs increased during the study period compared with the previous survey (Rüdelsheim & Smets, 2014); these were predominantly in Canada and the USA. Potato was trialled mostly in the USA and Europe, as was sugar beet, whereas most rice CFTs were recorded in India.

Three categories of trees in CFTs were discerned: poplar/aspens and Eucalyptus (for timber and biofuel); fruit trees; and ornamental trees. Other species included vegetables and fruits, fodder crops, other cereals, other oil and starch crops, ornamentals, and model crops for research.

Most CFTs in the USA were of maize, followed by soya bean, cotton, wheat and oilseed rape. For Canada, wheat overtook oilseed rape to be the most trialled crop. In Europe, potato was the most widely trialled plant species, closely followed by maize. In Latin America, the ranking was identical to in the USA. In Mexico, maize CFT applications were suspended, even though most of the events were at the end of development and had been commercialised elsewhere. In South Africa, maize, cotton and soya bean predominated; local crops, such as cassava, cowpea, banana, rice and sorghum were also trialled in other African countries. In Asia, rice was the most important crop in terms of number of CFTs, followed by maize and cotton; CFTs of all three crops were predominantly located in India. Other crops trialled in Asia were vegetables, especially aubergine (brinjal). Australia mainly trialled cotton, followed by oil crops (oilseed rape, Indian mustard and safflower) and cereals (barley and wheat). No maize CFTs were undertaken in Australia.

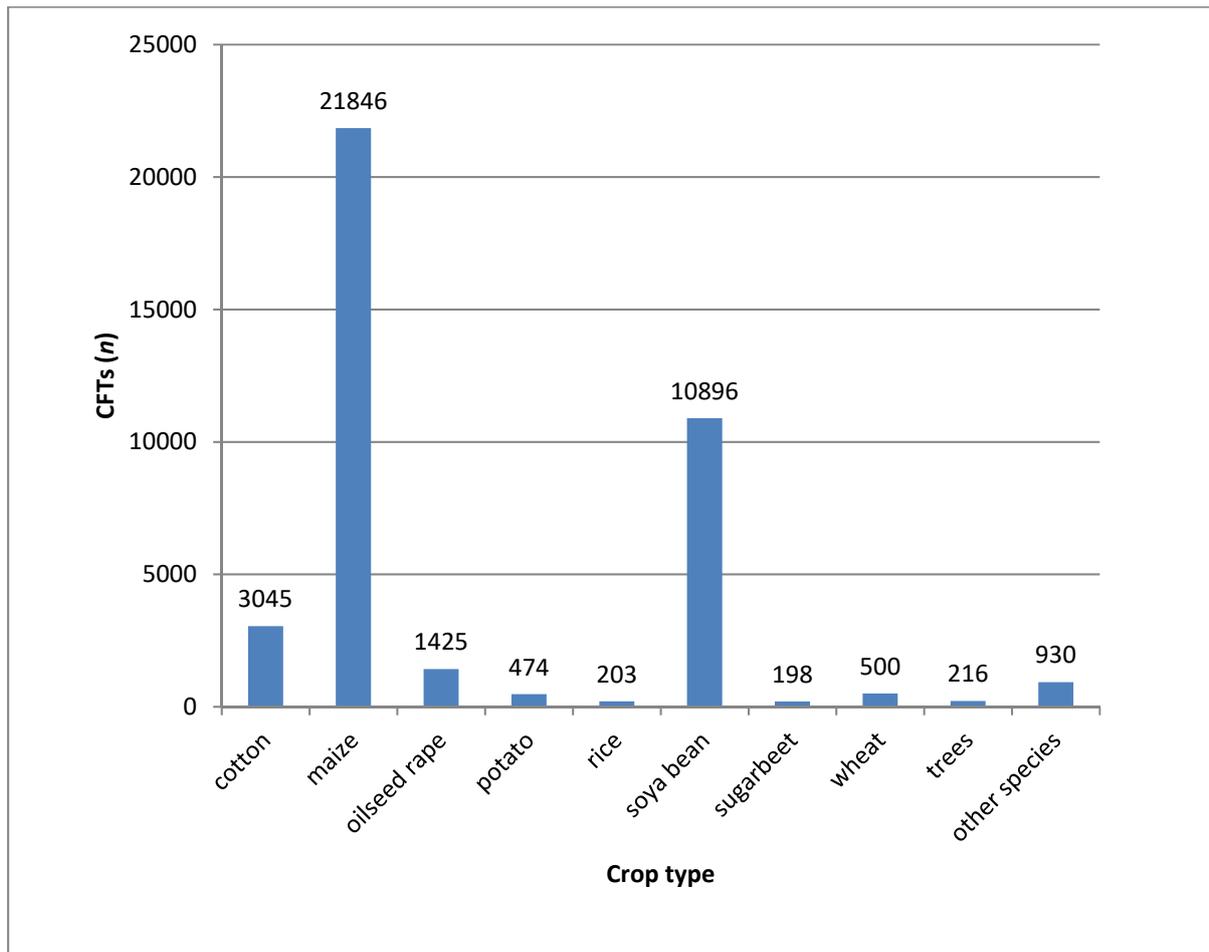


Figure 2. Total number of field trials per crop, 2014–2017

4.3. Traits

Figure 3 shows the distribution of distinct traits: the data derive from a total of 32,018 trials for which trait information could be retrieved. Trials performed in Chile were excluded because little or no trait information was available. Trait percentages were calculated as the percentage of trials for a certain trait. As it was possible to include several traits in a single trial, the sum of all percentages exceeds 100%.

Overall, herbicide tolerance was by far the most reported trait: more than 80% of all trials included GM plants containing a herbicide tolerance gene (83.9%). Plant biology traits (e.g. yield increase, biomass increase, plant architecture, fertility, growth rate) were reported in almost half of the trials (47.1%), while other agronomic properties such as abiotic stress tolerance (e.g. of drought, salt, heat, cold, frost and low nutrients) was reported in 37.1% of the trials. Both trait types increased compared with the previous survey (Rüdelsheim & Smets, 2014). For biotic stress resistance, insect resistance was the most common trait (54.9%) trialled, while product quality CFTs (21.9%) included traits concerning the type and level of fatty acids, carbohydrates and other compounds. Less than 1% of CFTs involved plants used as a production system for pharmaceutical and industrial proteins.

The number of marker gene CFTs (58.9%) was underestimated because these genes were often not reported in the databases. Conversely, it was not always clear whether herbicide tolerance genes were deliberately added to provide herbicide tolerance in the commercial product or merely to assist as selectable markers for identifying GM plants during the *in vitro* developmental phase; thus, the number of CFTs might be overestimated.

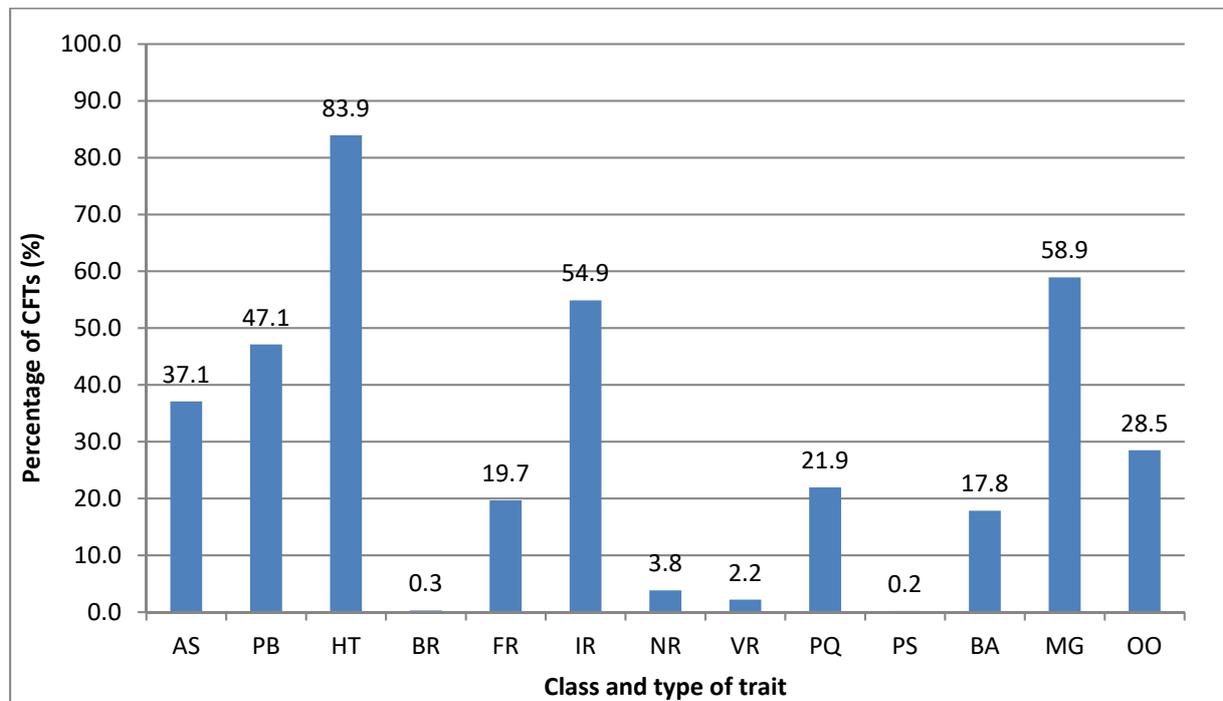


Figure 3. Percentage of all trials by class and type of trait, 2014–2017

AS: abiotic stress tolerance; BA: breeding aids; BR: bacterial resistance; FR: fungus resistance; HT: herbicide tolerance; IR: insect resistance; MG: marker genes; NR: nematode resistance; OO: other; PB: plant biology; PQ: product quality; PS: product systems; VR: virus resistance.

4.4. Traits Per Crop

Trait preferences can be detected when broken down per crop (Fig. 4). Again, herbicide tolerance was the major trait for the main commodity crops. For potato, this was exceeded only by fungal resistance (73% of all CFTs) owing to *Phytophthora infestans* being a major pest of this crop. Insect resistance remains important for cotton, maize and soya bean. Along with CFTs for GMOs with these well-established biotechnology traits, the number of CFTs with traits that favour crop yield continued to increase (included in PB). Abiotic stress tolerance (which stabilises yield) was trialled in most crops, especially maize (59% of all CFTs). Product quality traits were important for soya bean and oilseed rape (altered fatty acid composition) and potato (modified starch composition). For sugar beet, virus resistance (*Beet necrotic yellow vein virus*) was important, especially in Europe. In wheat, fungal resistance (e.g. *Fusarium*, rust) was the main trait trialled.

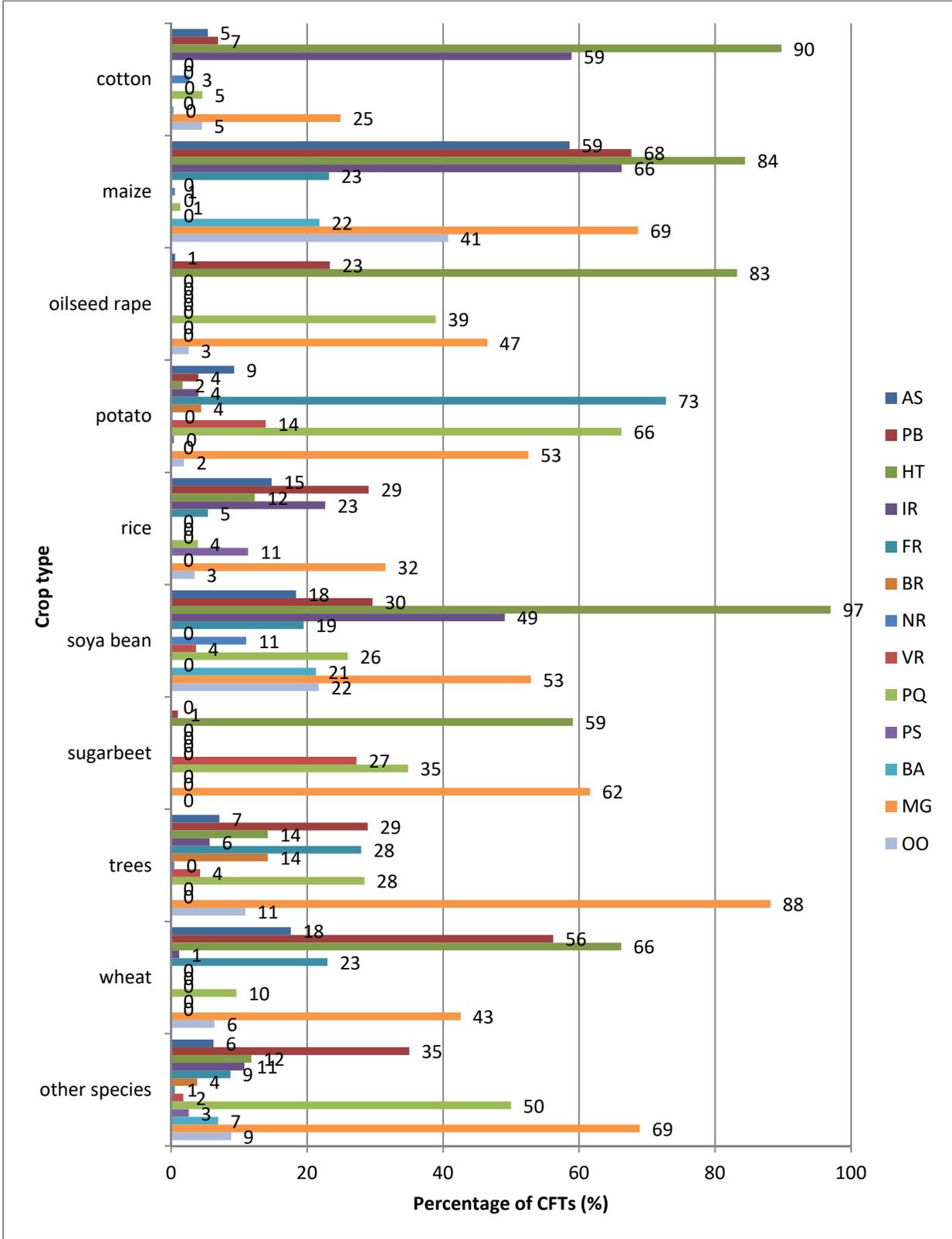


Figure 4. Percentage of all trials by crop, class and type of trait, 2014–2017

AS: abiotic stress tolerance; BA: breeding aids; BR: bacterial resistance; FR: fungus resistance; HT: herbicide tolerance; IR: insect resistance; MG: marker genes; NR: nematode resistance; OO: other; PB: plant biology; PQ: product quality; PS: product systems; VR: virus resistance.

In Africa, traits in commodity crops such as maize and cotton followed the global trends. However, the traits trialled in local crops clearly targeted specific local needs, e.g. bacterial resistance in banana, virus resistance in cassava, insect resistance in cowpea, abiotic stress tolerance in rice and both virus resistance and product quality in sweet potato.

4.5. Applicants

Two types of applicants quickly became apparent when collating the study data: industry and public research institutes. Research institutes were identified as not-for-profit research organisations, such as universities and government-owned institutes. They accounted for only 4.2% of all CFTs; in contrast, CFTs undertaken by industry accounted for 95.5% of all CFTs. Notably, research institutes usually acted locally, whereas industry comprised both multinational players and smaller enterprises that performed the trials in one or a few countries.

5. DISCUSSION

The large number of CFTs reported in this review is a consequence of the method used to determine the basic unit: every location combined with a trial year, when corrected for events tested at the same location at the same time, was considered an individual trial, even if separate notifications were made for the trial. For additional issues, care must be taken when interpreting these results:

- not all trial applications resulted in an actual CFT; and
- the availability of data was not uniform across countries.

In general, the annual number of CFTs worldwide declined over the study period although regional differences were observed: in North America and Europe, the number of trials decreased drastically, while only a slight decrease was observed in Latin America. Only in Australia did the annual number of CFTs remain largely constant during the study period. Unfortunately, the numbers for Africa and Asia were incomplete; thus, no trend could be observed.

Maize was the most widely trialled crop, and soya bean, cotton and oilseed rape continued to follow in importance, with wheat beginning to make inroads; however, regional differences were observed. By accounting for 95% of all CFTs, the major commodity crops continue to predominate. Although it is difficult to speculate on the underlying reasons, there are probably different influencing factors:

- Big markets justify the enormous investments that are required for the development and regulatory approval of GM crops. Lesser, niche GM crops may not present the same financial opportunities;
- Global commodities actually require a global programme with many repeated local CFTs. In such cases, it is likely that products that have already been approved in

one market enter into regulated CFTs in other potential markets, thereby making numerous appearances in multiple locations during the review period;

- GM products have been approved for most of the main arable crops, thus a regulatory track has been established for each. It is therefore more attractive to develop follow-on products for those crops in locations where a GM pipeline already exists;
- Companies with a global network and experience in bringing products to market are better placed to exploit CFT regulatory infrastructures.

For minor crops, the diversity remains the same and the number of CFTs is very low. Regulatory hurdles may discourage smaller developers from conducting a CFT, in particular those with a purely research objective. It is also possible that for minor crops and/or developments in local markets, fewer CFTs are required for market introduction. Further, for crops with a longer generation time (such as trees), new R&D activities might advance at a slower rate; thus, such crops might undergo CFTs at a lower frequency.

Herbicide tolerance remains the most trialled agronomic trait, and insects are the most targeted pest by GM crops. This does not mean, however, that only these first-generation traits are successful, for the following reasons:

- First-generation traits are further deployed beyond the primary markets. In the new markets, crops with these traits will probably fall under regulation and still require CFT approvals, and thus will still be included in this study. An example is an insect-resistant GM cotton that, after market approval in the USA, is further deployed in all essential cotton markets;
- In addition, seed production for export (e.g. counter-season production in Chile, Uruguay, etc.) contribute to the increased presence of first-generation traits in the study because the products will require field trial permits in the production countries;
- Some improvements in the trait type (e.g. stacking and combinations of different insect resistance modes of actions) may be overlooked because they all relate to the same trait and thus may not be reported separately;
- Given the success of the first-generation traits, it seems likely that new traits/products will be offered in combination with them. Therefore, if a new GM stacked event is to be trialled, the CFT will also include the proven herbicide tolerance and/or insect resistance trait.

Moreover, new traits are likely to be registered under the same trait type. For example, herbicide resistance not only includes resistance to glyphosate, glufosinate ammonium or sulfonyleureas but also to 2,4-dichlorophenoxyacetic acid (known as 2,4-D), dicamba and 4-hydroxyphenylpyruvate dioxygenase (known as HPPD) inhibitors, for example.

Other pest and disease resistance traits are investigated to a much lower extent and are greatly dependent on the host plant species. There is a growing number of field

trials for agronomic traits (plant biology traits, 47%; abiotic stress tolerance, 37%). Abiotic stress tolerance (mainly drought tolerance and nitrogen use efficiency) is the predominant traits in CFTs in the USA, where the main centres of research and GM crop development are located. Thus, new traits are likely to be first trialled in the USA, even if the products are ultimately intended for global commercialisation.

The next most important trait type is product quality (present in 22% of CFTs); however, over the study period this trait type began to lose ground. Lastly, an analysis of marker genes for selection (although not often reported in databases) revealed that antibiotic resistance markers and herbicide tolerance genes continue to be widely used.

Industry is the primary applicant for CFTs of the main crops, whereas lesser crops tend to be developed mainly by universities or government research institutes. In Asia and Africa, the number of CFT applications submitted by public research centres and public–private partnerships is increasing. The following list illustrates a phased establishment of a GMO pipeline.

1. The fastest way to introduce a GM crop is the local adaptation of an elite event that has already been developed for a primary market. A good example is insect-resistant cotton, which was developed and marketed in the USA and then adapted in Burkina Faso for the local market.
2. More local involvement is required when the initial transformations, selection and testing (usually involving some research CFTs) is done abroad, whereas the development CFTs and preparation for commercial release are performed locally. Examples include banana and cassava projects linking North American and Australian researchers with African agronomic centres.
3. Establishing the complete R&D infrastructure requires that molecular and plant transformation activities, which are the critical start for development projects, are carried out in local laboratories.

While acknowledging the challenges and limitations of the study approach to creating a standardised inventory of CFTs of GM plants, the overall numbers that have been performed over the years is huge. The inventory to date holds records of nearly 161,000 CFTs reported since 2001. The recorded CFTs span a wide range of countries, crops and traits; were conducted by both public and private organisations; and have been subjected to regulatory oversight. This broad experience should provide an ample basis for regulatory systems to streamline their processes and procedures so that lesser and more local crops with financially less attractive traits can also progress safely to market.

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