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Current and future market applications of new genomic techniques

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Contents

Abstract

This report presents a review of market applications of new genomic techniques (NGTs). For the purposes of this study, NGTs are defined as 'techniques that are able to alter the genetic material of an organism, developed after the publication of EU Directive 2001/18/EC'.

The study covers NGT applications in agri-food, industrial and medicinal sectors that have resulted in applications that are already being marketed, are at a confirmed pre-market development stage or are at a research and development (R & D) stage but showing market potential. The scope includes the use of NGTs in any kind of plant, mushroom, animal or microorganism or in human cells.

Data on NGT applications were collected from multiple sources, including information available online, consultation of experts and an ad hoc survey of public and private technology developers. The NGT applications identified were classified, using the information available, as being at the following development stages.

- **Commercial stage.** NGT applications currently marketed in at least one country worldwide.
- **Pre-commercial stage.** NGT applications ready to be commercialised in at least one country worldwide but not yet on the market (commercialisation mainly depends on the developer's decision and a 5-year horizon is estimated).
- **Advanced R & D stage.** NGT applications at a late stage of development (field trials in the case of plants, *in/ex vivo* clinical trials in the case of medical applications) and likely to reach the market in the medium term (i.e. by 2030).
- **Early R & D stage.** NGT applications at proof of concept stage (i.e. testing gene targets for trait enhancement of commercial interest).

NGTs, especially those based on clustered regularly interspaced short palindromic repeats (CRISPR), are being actively and increasingly used in all the sectors analysed. Currently, few NGT applications are marketed worldwide: one plant product, one microorganism for release into the environment and several microorganisms used for contained production of commercial molecules. There are, however, about 30 identified applications (in plants, animals and microorganisms) at a pre-commercial stage in the pipeline that could reach the market in the short term (within 5 years). In addition, the medicinal sector is actively using NGTs to tackle several human diseases, and in many cases applications have already reached patients, in phase I and phase I/II clinical trials.

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Executive summary

This study reviews market applications of new genomic techniques (NGTs). For the purposes of this study, NGTs are defined as 'techniques that are able to alter the genetic material of an organism, developed after the publication of EU Directive 2001/18/EC'. The study, requested by the European Commission's Directorate-General for Health and Food Safety from the Joint Research Centre, is part of a broader study that the European Commission prepared on this topic at the request of the European Council (https://ec.europa.eu/food/plant/gmo/modern_biotech/new-genomic-techniques_en).

NGTs include the following four groups of techniques, as explained by Broothaerts et al. (2021): (1) techniques that create a double strand break (DSB) in the DNA, including site-directed nuclease (SDN) techniques based on clustered regularly interspaced short palindromic repeats (CRISPR), transcription activator-like effector nucleases (TALEN), zinc-finger nucleases (ZFN) and homing endonucleases, which techniques can lead to mutagenesis and some of them also to cisgenesis, intragenesis or transgenesis; (2) techniques that involve a single strand DNA (ssDNA) break or no break at all in the genome, such as oligonucleotide-directed mutagenesis (ODM), base editing and prime editing; (3) epigenetic techniques such as RNA-directed DNA methylation (RdDM) and CRISPR interference (CRISPRi); and (4) techniques that act directly on RNA (RNA editing). Conversely, techniques already in use prior to 2001, such as *Agrobacterium*-mediated techniques or gene guns, are not considered NGTs.

The study covers NGT applications in agri-food, industrial and medicinal sectors that have resulted in products that are already being marketed, are at a confirmed pre-market development stage or are at a research and development (R & D) stage but showing market potential. The scope includes the application of NGTs in any kind of plant, mushroom, animal or microorganism or in human cells.

Data on NGT applications ([1]) were collected from multiple sources, including information available online, consultation of experts and an ad hoc survey of public and private technology developers.

The NGT applications identified were classified, using the information available, as being at the following development stages.

- **Commercial stage.** NGT applications currently marketed in at least one country worldwide.
- **Pre-commercial stage.** NGT applications ready to be commercialised in at least one country worldwide but not yet on the market (commercialisation mainly depends on the developer's decision and a 5-year horizon is estimated).
- **Advanced R & D stage.** NGT applications at a late stage of development (field trials in the case of plants, *in*/*ex vivo* clinical trials in the case of medical applications) and likely to reach the market in the medium term (i.e. by 2030).
- **Early R & D stage.** NGT applications at proof of concept stage (i.e. testing gene targets for trait enhancement of commercial interest).

Data are reported in this document by organism group (plants, mushrooms, animals, microorganisms and human cells) and the figures illustrate trends in the sectors analysed. The data can also be consulted using the web dashboard at this link: https://datam.jrc.ec.europa.eu/datam/mashup/NEW_GENOMIC_TECHNIQUES.

General picture of the NGTs currently being used

According to the data collected, Group 1 NGTs are currently the most used (almost 91 % of NGT applications). Within Group 1, CRISPR-based techniques clearly dominate the scene. According to the experts consulted, CRISPR is cheaper, quicker and more efficient than the other techniques. In most applications, CRISPR/Cas (and other SDN systems) are used to obtain small mutations/insertions through non-homologous end joining (SDN1), while their use with a DNA template (SDN2 and SDN3) has been much less fully exploited so far.

Group 2 NGTs (ODM and others) were used in about 7 % of the applications identified in this study. No more than 10 applications were identified, at R & D levels, that used NGTs in Group 3 (epigenetics) or Group 4 (RNA editing).

([1]) For the purpose of this study, the term 'application' refers to organisms in which an NGT is applied to obtain a trait of interest. It has no regulatory implications.

Regarding the countries in which NGT applications have been developed, the United States and China are the most active ones. Within the European Union, Germany was the country of origin of the largest number of applications identified in this study. Thanks to the flexibility and affordability of NGTs (especially those based on CRISPR), several developing countries are also active in the field.

Regarding the distribution of private and public/academic developers, both groups are active. However, commercial and pre-commercial applications from private companies are more numerous, while public/academic organisations are more present in the R & D stages, where they are contributing to the creation a very rich pipeline in terms of variety of organisms and traits.

Plants

In this study, we identified 427 applications of NGTs in plants, in many species. Only one plant application was found to be on the market in at least in one country worldwide: a soybean variety with a high oleic acid content, modified with TALEN (a Group 1 NGT) ([2]).

We classified 16 plant applications as being at the pre-commercial stage, including some plant–trait combinations already developed with established genomic techniques, such as maize, soybean, rice and potato with traits such as herbicide tolerance, fungal resistance, modified oil or starch composition and non-browning properties. Other applications have not been reported before, such as tomato fortified with the dietary supplement gamma-aminobutyric acid (GABA), herbicide-tolerant pigeon pea and flax, and pennycress and camelina with modified oil content.

The sizes of the advanced R & D stage (117 plant applications) and the early R & D stage (292 plant applications) show the potential of NGT plants in the medium term (by 2030) and the diversity of applications in terms of traits and plants. Disease-resistance traits target many types of pathogens and pests. Abiotic stress tolerance is widely represented in the data collected and includes tolerance to drought, salinity and heat. Modified composition goes beyond starch and oil content, and many applications are emerging in crops with better nutritional properties (fibre or vitamin content) as well as in reducing potentially harmful properties (toxins, allergens, acrylamide, etc.) or gluten. Furthermore, several applications are dedicated to obtaining higher and/or more stable yield (in terms of plant production and/or size of fruits or grains).

Mushrooms

For mushrooms, only one NGT application was identified: a non-browning white button mushroom (*Agaricus bisporus*), obtained with CRISPR/Cas9, considered to be at the pre-commercial stage.

Animals

The data collected show that NGTs in animals are mainly used for food purposes, in particular in farm animals such as cattle, pigs and chicken, and in various fish (salmon, tilapia, tuna and red sea bream). Insects (especially mosquitos) and some invasive species are the subjects of NGT-based gene drive applications.

No NGT animals have yet been commercialised, while four animals are at the pre-commercial stage (yield-enhanced/fast-growing tilapia, porcine reproductive and respiratory syndrome-resistant pigs, hornless cattle and heat-resistant cattle). The advanced and early R & D stages show a total of 59 identified NGT applications, with food-related traits predominating, followed by gene drive applications.

One particular use of NGTs in animals is in the field of research (advanced and early R & D) on human diseases, using animals as disease models to search for a gene therapy or using animals to produce organs that can be transplanted into human patients. So far, mice are the model animals in which NGTs are most commonly applied in human gene therapy studies (in particular in studies on cancer and genetic diseases). Pigs will be particularly important for the future production of organs that do not cause transplant rejection in humans. NGT-modified rats and monkeys are also used to model human diseases, but this application is still at the early R & D stage.

[2] Please, note that, after the finalisation of this report, we learned that one pre-commercial plant application (CRISPR/Cas-derived GABA tomato) moved further towards commercialisation and will soon be cultivated in Japan.

Microorganisms

According to the experts consulted, NGTs are already applied commercially in microorganisms for the contained bio-production of industrial molecules, in which the NGT microorganism is used as a bio-factory and not as the final market product. In this case, technology developers continuously use genetic techniques (including both established and new genomic techniques) to improve a specific strain, usually with a long history of efficiency as a bio-factory and safe use, until they reach the desired goal.

In this strain improvement context, the most commonly used NGTs belong to Group 1 (in particular those based on CRISPR) and are used to knock out unfavourable genes, encoding, for example, for toxins, intrinsic antibiotic resistance or by-products. According to the experts consulted, NGTs have great potential in relation to future microorganism strain improvement and are becoming standard tools in some cases. However, it is difficult to estimate the current share of microorganism strains used by bio-industry worldwide that have been improved with NGTs.

Another market sector is the commercialisation of NGT microorganisms as final products for release into the environment. Here, we identified one commercial NGT application in soil bacteria for fertilisation of agricultural soils. The R & D pipeline includes other examples of applications in soil bacteria and in probiotics.

Human health

NGTs are widely employed in medical/therapeutic applications focusing on human diseases, including uses in human cells *in vitro*, *in vivo* and *ex vivo*.

Some NGT applications for human health have gone beyond pre-clinical research and are reported in 63 identified clinical trials, which are at either phase I or phase I/II. Data from literature also reveal significant activity at the early R & D stage, focused on a similar list of conditions.

Cancer is the main target of therapeutic applications involving the use of NGTs, with 48 applications, plus 8 applications relating to types of cancer caused by viral infections. The target with the second highest number of applications is viral diseases (23 applications), and many applications target hereditary diseases, including haematological diseases (16 applications), some neurodegenerative diseases (2 applications for Huntington's disease), eye diseases (5) and other hereditary diseases (8). In terms of types of human cells targeted, the highest number of identified applications target T cells (mostly autologous but in some cases allogenic), followed by stem cells and cancerous cells.

In addition, thanks to the flexibility of CRISPR, both in terms of sequence specificity and different uses, it is already being applied to the search for solutions for rapid detection of coronavirus disease 2019 and also in some therapeutic options against the disease.

Conclusion

NGTs, especially those based on CRISPR, are being actively and increasingly used in all the sectors analysed. Applications at market and pre-market stages are still few, but the R & D pipeline is very rich. The reasons for this may relate to the fact that NGTs (especially those based on CRISPR) are still a recent discovery and/or to regulatory uncertainty about these techniques in several countries. Thanks to its flexibility, affordability and ease of use, CRISPR is opening the doors to several new possibilities, and many more applications are expected to appear in the future and eventually to reach the market.

1. Introduction

This study reviews current and future market applications of new genomic techniques (NGTs). The study, requested by the European Commission's Directorate-General for Health and Food Safety from the Joint Research Centre (JRC), is part of a broader study that the European Commission prepared on this topic at the request of the European Council (https://ec.europa.eu/food/plant/gmo/modern_biotech/new-genomic-techniques_en).

This review provides insights into and describes the state of the art in applications of NGTs in agri-food, industrial and medicinal sectors, based on data on:

- products using NGTs already marketed worldwide;
- products using NGTs that are at a pre-market development stage (including experimental releases) worldwide.

For the purposes of this study, NGTs are defined as 'techniques that are able to alter the genetic material of an organism, developed after the publication of Directive 2001/18/EC', the EU directive on genetically modified organisms (GMOs) (European Parliament and Council, 2001). Genomic techniques used before 2001, for example transformation through *Agrobacterium* or biolistic techniques, are therefore excluded from the scope of the study.

As explained by Broothaerts et al. (2021), NGTs include the following four groups of techniques: (1) techniques that create a double-strand break (DSB) in the DNA, including site-directed nuclease (SDN) techniques such as those based on clustered regularly interspaced short palindromic repeats (CRISPR), transcription activator-like effector nucleases (TALEN), zinc-finger nucleases (ZFN) and homing endonucleases, which techniques can lead to mutagenesis and some of them also to cisgenesis, intragenesis or transgenesis; (2) techniques that involve a single-strand DNA (ssDNA) break or no break at all in the genome, such as oligonucleotide-directed mutagenesis (ODM), base editing and prime editing; (3) epigenetic techniques such as RNA-directed DNA methylation and CRISPR interference (CRISPRi); and (4) techniques that act directly on RNA (RNA editing). Conversely, techniques already in use prior to 2001, such as *Agrobacterium*-mediated techniques or gene guns, are not considered NGTs.

The scope of the study covers applications in agri-food, industrial and medicinal sectors that have resulted in products that are already being marketed or are at a confirmed pre-market development stage, including experimental releases. The scope includes any kind of plant, mushroom, animal or microorganism or human cells altered with NGTs.

The use of NGTs in these organisms for fundamental research purposes, such as technology development (e.g. new/improved genome editing tools), gene discovery research or the creation of human disease models in animals (e.g. to provoke cancers, metabolic disorders or other typically human diseases in animals), is outside the scope of this study.

2. Methodology

The data collection on market/near-market applications of NGTs in the different organisms and sectors was carried out through:

- the creation of a database of NGT applications, based on the information available online;
- consultation of experts in the NGT field through videoconferences and written communication;
- a survey of public and private technology developers;
- integration and cleaning of the data from different sources in the database;
- determination of the development stage (market, near-market or research and development (R & D)) of the applications identified.

Each step is described further in the following sections.

2.1. Database of new genomic technique applications

The database of NGT applications was created in an Excel file with the following headings, under which the information specified was added for each application.

- **Application ID.** An identification code assigned for the purpose of the study.
- **Source of information**. Indication of the dataset, expert or other source from which the information originated.
- **Technique**. The technique used, in general terms: a CRISPR platform, TALEN, ZFN, etc.
- **Technique details**. More details on the specific NGT employed, e.g. 'CRISPR/Cas9'.
- **Technique group (1, 2, 3, 4)**. Following the classification into four groups established in Broothaerts et al. (2021).
- **SDN type (1, 2, 3)**. The type of SDN technique used, where applicable ([3]).
- **Organism group**. Plant, mushroom, animal, human, microorganism.
- **Species group.** Within the same organism group, species with similar characteristics were aggregated into categories (e.g. cereals, fruits, aquatic animals, insects, etc.).
- **Species.** The specific organism in which the technique is used (e.g. potato, cattle, bacteria, etc.).
- **Commercial name.** Where available.
- **Country.** Country of the developer (or of the clinical trials in the case of human health applications).
- **Company/institution.** Name of the developer.
- **Trait / disease description.** Description of the specific trait or disease for which the NGT is employed.
- **Trait / disease category.** Category of trait or disease for which the NGTs is employed, as described in each specific section.
- **Designation / target gene.** If available, name of the gene targeted by the NGT.
- **Development stage.** Indication of the proximity to market of the product/application, as explained in **Section 2.5**.

([3]) ZFN, TALEN, Meganuclease and Cas are SDN techniques.
SDN1 mechanisms involve small deletions/insertions caused by the DNA repair process through non-homologous end joining after the generation of DSBs by the nucleases.
SDN2 mechanisms involve changes to one or more base pairs through homologous recombination by copying a repair template delivered together with the nucleases as a consequence of the DSB.
SDN3 mechanisms involve the site-specific insertion of a DNA stretch delivered together with the nuclease, through homologous recombination following the DSB.

- **Reference.** Specific link or reference to the web page or article in which the information was reported.
- **Application method.** For human health applications, if the method is *in vitro*, *in vivo* or *ex vivo*.

Since much of the data was obtained under conditions of confidentiality, the report shows data aggregated into species groups and trait/disease categories. The detailed content of the database will not be made public.

The sources of information consulted to build the database of NGT products were:

- the Genetic Literacy Project (https://crispr-gene-editing-regs-tracker.geneticliteracyproject.org/);
- the Julius Kühn Institute (see Menz et al., 2020)
- the Animal and Plant Health Inspection Service of the US Department of Agriculture (USDA) (https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/am-i-regulated/regulated_article_letters_of_inquiry/regulated_article_letters_of_inquiry);
- Innovature (https://innovature.com/);
- the European Association for Bioindustries (EuropaBio) (http://test.europabio.org/cross-sector/publications/genome-editing-%E2%80%94-what-if-we-embraced-its-potential);
- CropLife (https://croplife.org/agricultural-innovation/);
- National Geographic (https://www.nationalgeographic.com/environment/future-of-food/food-technology-gene-editing/);
- Progressive Agrarwende (https://progressive-agrarwende.org/crispr-adventskalender-blog/);
- US National Science Foundation Plant Genome Editing Database (http://plantcrispr.org/cgi-bin/crispr/index.cgi);
- Labiotech.eu (https://www.labiotech.eu/crispr/crispr-applications-gene-editing/);
- several articles, blogs and press releases, as indicated in the Excel database for each application;
- additional scientific literature, as indicated in the Excel database for each application.

For applications of NGTs for human health, the following databases of clinical trials were also consulted, through a search based on the keywords 'CRISPR', 'TALEN' and 'ZFN' (which the literature indicated were the three NGTs used in clinical trials):

- the EU Clinical Trials Register (https://www.clinicaltrialsregister.eu/ctr-search/search/),
- the EU Summary Notification Information Format (SNIF) database (https://gmoinfo.jrc.ec.europa.eu/),
- the US ClinicalTrials.gov database (https://clinicaltrials.gov/),
- the World Health Organization International Clinical Trials Registry Platform (https://apps.who.int/trialsearch/),
- the Chinese Clinical Trial Registry (http://www.chictr.org.cn/abouten.aspx),
- the Japanese University Hospital Medical Information Network Clinical Trials Registry (http://www.umin.ac.jp/ctr/), the Japan Medical Association Center for Clinical Trials Clinical Trial Registry (http://www.jmacct.med.or.jp/en/what-we-do/registry.html) and the Japan Pharmaceutical Information Center Clinical Trials Information platform https://www.clinicaltrials.jp/cti-user/trial),
- the Australian New Zealand Clinical Trials Registry (https://www.anzctr.org.au/TrialSearch.aspx),
- the South Korean Clinical Research Information Service (https://cris.nih.go.kr/cris/en/search/basic_search.jsp),
- the Indian National Institute of Medical Statistics Clinical Trials Registry (http://ctri.nic.in/Clinicaltrials/advancesearchmain.php).

2.2. Consultation of new genomic technique experts

For the integration and validation of the data collected, several experts were identified worldwide, belonging to the following three groups:

- experts from governmental organisations in charge of biotechnology regulation,
- private technology developers,
- public technology developers.

Most of these experts were contacted by email and invited to videoconferences (using the Cisco Webex platform) with representatives of the JRC and the Directorate-General for Health and Food Safety. The agenda for these videoconferences was as follows.

- Introduction from the Directorate-General for Health and Food Safety about the European Commission's larger NGT study.
- Introduction to the JRC and its role.
- Presentation by the JRC about its tasks in the framework of the study and its scope, objectives and methodology.
- Introduction from the invited expert(s), with focus on current activities related to applications of NGTs in their field.
- Interaction between the JRC and the invited expert(s) about the possibility of data sharing to support the JRC data collection.
- Timeline for further steps.

Confirmed participants received the proposed agenda ahead of the meeting and were informed that the purpose of the meeting was purely related to the JRC data collection exercise on NGT applications and not to discuss regulatory/policy issues.

Additional information about data sources was received from the experts consulted through email and/or brief telephone calls.

The list of experts consulted covers most key countries in the development of NGT applications. Several attempts were made to get in touch with experts from China, but unfortunately without success. A list of the organisations of the experts consulted is provided below.

Regulators:

- the USDA
- the Office of the Gene Technology Regulator (OGTR), Australia
- Food Standards Australia New Zealand (FSANZ)
- the Environmental Protection Authority, New Zealand.

Private developers:

- EuropaBio
- Euroseeds
- the European Forum of Farm Animal Breeders (EFFAB)
- the European Federation of Pharmaceutical Industries and Associations (EFPIA)
- Novozymes A/S, Denmark
- the American Seed Trade Association (ASTA), United States
- the International Seed Federation (ISF), United States
- Biogen Idec Ltd, United Kingdom
- Corteva, United States

- Bayer, Germany
- BASF, Germany
- Inari, United States
- ChileBio, Chile
- the Alliance for Regenerative Medicine (ARM)
- Lallemand and Mascoma LLC (subsidiary of Lallemand)
- the Pharmabiotic Research Institute (PRI)

Public/academic developers:

- the European Plant Science Organisation (EPSO)
- the Network of European Sustainable Agriculture through Genome Editing (EU-SAGE)
- the Farm Animal Breeding and Reproduction Technology Platform (FABRE TP)
- the Julius Kühn Institute, Germany
- the National Agriculture and Food Research Organization (NARO), Japan
- Universidad Nacional de Quilmes, Argentina
- the Consultative Group on International Agricultural Research (CGIAR).

2.3. Survey of technology providers

In most cases, the experts consulted agreed to help with the JRC data collection on NGT applications by filling in a questionnaire prepared by the JRC. Representatives of associations of industries or research institutes agreed to send the JRC questionnaire to their members. In total, 47 organisations participated in the survey, including 37 private companies and 10 public/academic organisations, representing several countries worldwide, both developed and developing countries (a list is not provided for confidentiality reasons). An example questionnaire with the accompanying background information is provided in Annex 1.

2.4. Data integration and cleaning

Data obtained from various sources, as described above, were merged into one Excel database. Duplicates (i.e. information regarding the same NGT application) were removed, and this served to validate and ensure the completeness of the information. Where there were data gaps, the missing information was searched for online. As most information derived from private organisations, some details were not publicly disclosed by the companies, to protect their business interests, and therefore some data gaps remained.

2.5. Development stages

The NGT applications identified were classified, using the information available, as being at the following development stages.

- **Commercial stage.** NGT applications currently marketed in at least one country worldwide.
- **Pre-commercial stage.** NGT applications ready to be commercialised in at least one country worldwide but not yet on the market (commercialisation mainly depends on the developer's decision and a 5-year horizon is estimated).
- **Advanced R & D stage.** NGT applications at a late stage of development (field trials in the case of plants, *in*/*ex vivo* clinical trials in the case of medical applications) and likely to reach the market in the medium term (i.e. by 2030).

- **Early R & D stage.** NGT applications at proof of concept stage (i.e. testing gene targets for trait enhancement of commercial interest).

The classification used was different for NGT human health applications. These are described in scientific papers or are in use in clinical trials at phase I or phase I/II (i.e. no commercial or pre-commercial applications). Applications in patients (*in vivo* and *ex vivo*, as explained in **Section 4.4**), which included most of the applications in clinical trials, were classified as at the advanced R & D stage. *In vitro* applications (described in most of the scientific papers) were classified as at the early R & D stage.

2.6. Data analysis and web dashboard

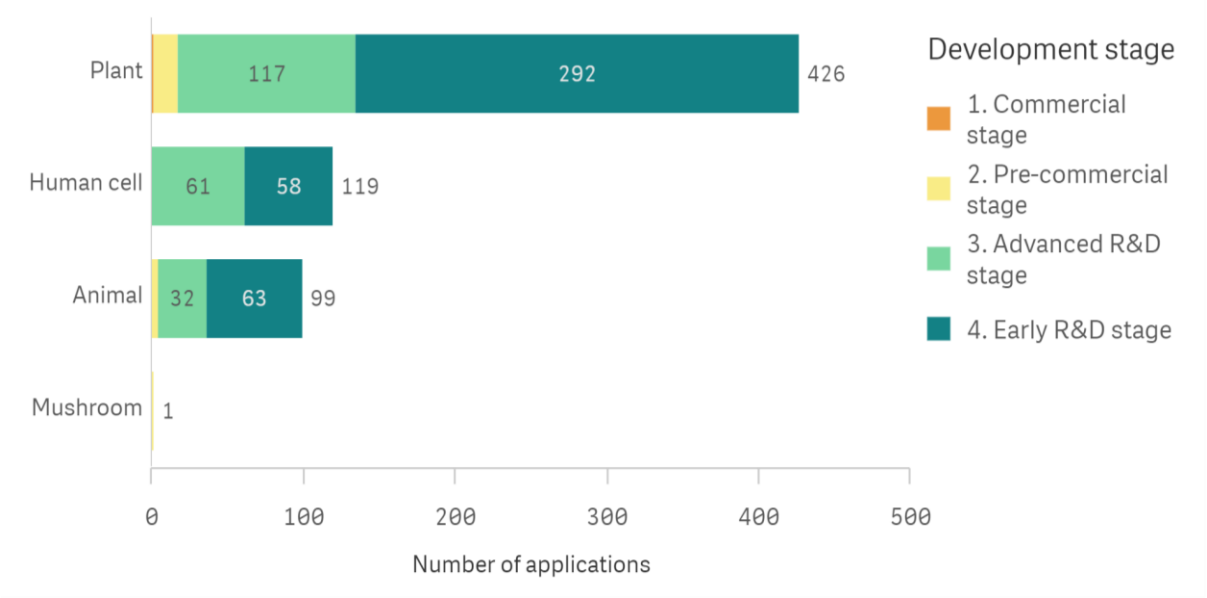
The data collected are described in the following sections, organised by organism group (plants, mushrooms, animals, microorganisms and human cells). The data are illustrated in figures showing trends in the sectors analysed in terms of most used NGTs, most active countries, types of developers, species, traits and development stages. The data can also be consulted using the web dashboard at this link: https://datam.jrc.ec.europa.eu/datam/mashup/NEW_GENOMIC_TECHNIQUES, where several graphs are presented and the user can filter for the characteristics in which he or she is interested.

3. Market application review of new genomic techniques

The following sections describe the results obtained for the five types of organisms included in the scope of the study: plants, mushrooms, animals, microorganisms and human cells. As explained in the corresponding section, data on NGT-derived microorganisms were not aggregated and illustrated in the same way as data for the other types of products, because of a different data structure and more limited availability.

Figure 1 provides a general overview of the 645 NGT-produced plants, mushrooms, animals and human cells identified in the study, by development stage. Data on microorganisms are not shown in the figures because of the specificities of the use of NGTs for microorganisms and owing to data gaps (see **Section 4.4.2**).

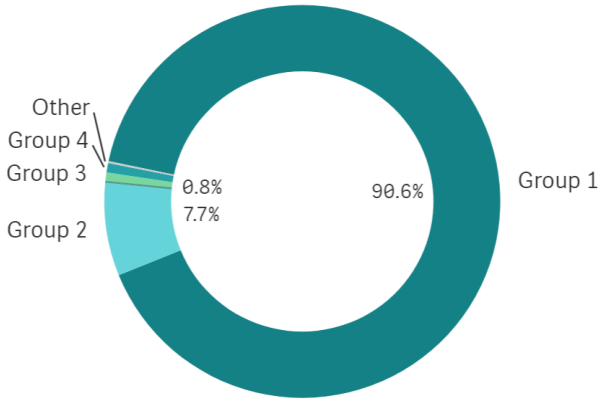
Figure 1. NGT-produced plants, mushrooms, animals and human cells, by development stage



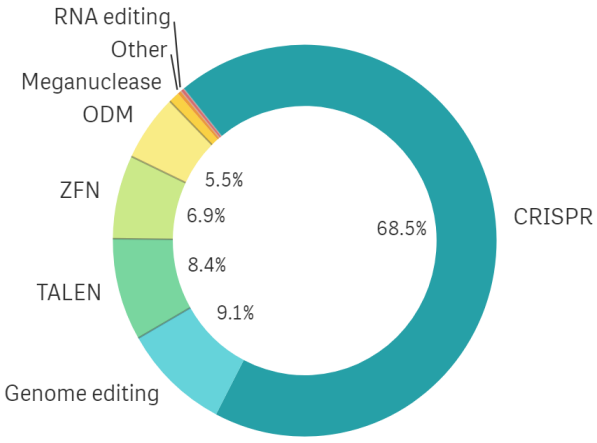
Source: Authors' research.

Figure 2. NGTs employed in plants, mushrooms, animals and human cells identified in this study, classified according to the technique group to which they belong (see Broothaerts et al., 2021) and the technique used.

2a: Technique groups



2b: Techniques



NB: 'Genome editing' refers to products for which no further specifics were provided.

Source: Authors' research.

Before we analyse the characteristics of each application sector, the following general observations can be made on the data.

- Among the four groups of techniques identified by Broothaerts et al. (2021), Group 1 techniques are currently the most used (almost 91 % of NGT applications) in products with market potential, followed by Group 2 techniques, representing 7.7 % (see **Figure 2a**), mainly in plants. Group 2 NGTs (ODM and others) were used in about 7 % of the applications identified in this study. No more than 10 applications were identified, at R & D levels, that used NGTs in Group 3 (epigenetics) or Group 4 (RNA editing).
- Group 1 techniques, such as SDN techniques, are used predominantly to obtain small mutations/insertions through non-homologous end joining (SDN1), while their use with a DNA template (SDN2 and SDN3) has been much less fully exploited so far (data not shown).
- Among the different NGTs, CRISPR-based techniques clearly dominate the scene (see **Figure 2b**). According to the experts consulted, CRISPR is cheaper, quicker and more efficient than the other techniques.
- Currently, only one crop plant produced with NGTs has been identified as on the market worldwide, and no NGT-produced animal is yet on the market. However, we have identified about 20 NGT products (plants, animals and mushrooms) that could be considered to be at pre-commercial stage.
- In the case of NGT-produced microorganisms intended to be marketed as a final product, one commercial application has been identified. However, we can observe that NGTs are widely used in the production of commercial microorganism strains for the contained production of molecules of industrial interest (food, feed, energy, bio-based chemicals), especially to improve the producer strains. In these cases, the final commercial products are not NGT microorganisms but molecules produced by them.
- Further down the pipeline for market release, we have identified over 600 NGT-derived products that could be classified as at the advanced or early R & D stage, revealing significant commercially oriented activity and perceived potential in NGTs.
- The medicinal sector is very active in using CRISPR and other NGTs to tackle several human diseases, and some applications have already reached patients, in phase I and phase I/II clinical trials, but not yet at commercial or pre-commercial level.
- The reasons for this gap between commercial/pre-commercial and R & D-stage applications could relate to the fact that NGTs (especially CRISPR) are still a recent discovery or to regulatory uncertainty about these techniques in several countries.

3.1. Plants

The data collected on plants obtained using NGTs show a rich pipeline, with more than 400 applications in several types of plants, including arable crops, vegetables, fruits and also plants that usually do not receive a great deal of attention from developers and researchers because of smaller turnovers (so-called orphan crops such as cassava, millet, chickpea, etc.). This suggests the high degree of plasticity/flexibility of NGTs (especially those based on CRISPR) in a wide range of different organisms.

A summary of the plant groups and species in which NGTs are used is provided in **Table 1**. The list of traits obtained using NGTs in plants is also very diverse. A summary of the traits included in the database is provided in **Table 2**, where the traits are grouped into categories.

Table 1. Summary of plants identified in the database of NGT products

Plant groups	Plants included (not exhaustive)
Cereals	Maize, wheat, rice, barley, sorghum, millet
Forage and grasses	Alfalfa, ryegrass, switchgrass, *Setaria viridis*
Fruits	Apple, banana, orange, groundcherry, grapefruit, grapevine, kiwi fruit, melon, watermelon, berries, stone fruits, avocado
Legumes	Beans, chickpea, peanut, pea, pigeon pea
Oil and fibre crops	Soybean, rapeseed, cotton, camelina, flax, pennycress, sunflower, mustard, strawberry
Ornamentals	Chrysanthemum, dandelion, orchid, petunia, poinsettia, poppy, Japanese morning glory, wishbone flower, jasmine tobacco
Sugar crops	Sugar beet, sugar cane
Trees	Poplar, softwood trees
Tubers and root vegetables	Potato, sweet potato, cassava, beetroot
Vegetable crops	Tomato, broccoli, cabbage, cucumber, aubergine, lettuce, pepper, chicory
Plants (aggregated)	When only 'plants' or a list of various plants was identified
Other plants	Cacao, coffee, tobacco, salvia

Source: Authors' research.

Table 2. Trait categories identified in NGT-produced plants in the database

Trait category	Description
Biotic stress tolerance	Resistance to biotic stressors such as nematodes, fungi, bacteria, viruses and other pests, pathogens or parasites
Abiotic stress tolerance	Resistance to abiotic stressors such as drought, heat, salt, rain or ultraviolet radiation
Herbicide tolerance	Tolerance to various types of herbicides
Modified colour/flavour	Modified colour or flavour
Modified composition	Modified content of substances such as starch, oil, proteins, vitamins, fibres, toxic substances, allergens, etc., to improve food/feed quality or for a better industrial use (includes seedless fruits as a quality characteristic)
Plant yield and architecture	Yield increase (or yield stability) related to higher number of flowers/seeds/fruits, to fruit size/weight or to photosynthetic efficiency. Includes other changes in plant architecture like plant height and shape, fruit shape and growth pattern
Storage performance	Improvement of characteristics such as shelf life and tolerance of storage conditions (e.g. cold storage), including non-browning and reduced black spot
Other traits	Traits not classified in the above categories, including production of molecules of industrial interest, flowering time for agronomic purposes and nitrogen use
Breeding tools	Reproductive/flowering characteristics including induction of sterility, early flowering and haploid techniques

Source: Authors' research.

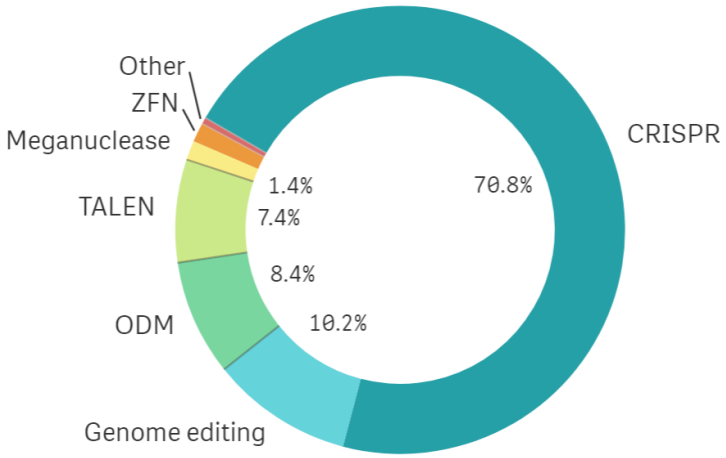
Of the 426 NGT-produced plants reported in the database, only one commercial plant has been identified so far: a high-oleic soybean variety modified with TALEN by the United States-based company Calyxt. TALEN was used to inactivate two genes involved in fatty-acid synthesis through targeted mutagenesis (Haun et al., 2014), resulting in oil with an improved nutritional profile owing to reduced content of saturated fatty acids. According to the available information, the TALEN-modified soybean is cultivated in the United States and the 'Calyno oil' derived from it is sold both in the United States and Canada ([4]).

Of the NGT-produced crop plants in our database, 16 were identified as being at the pre-commercial stage. Some crop–trait combinations found in this group have been obtained in the past with established genomic techniques (e.g. *Agrobacterium* or biolistics to obtain transgenic or cisgenic plants): these relate to crops such as maize, soybean, rice and potato, and traits such as herbicide tolerance, fungal resistance, modified oil or starch composition and non-browning (longer shelf life) properties. However, some new crop–trait combinations emerged, such as tomato fortified with the dietary supplement gamma-aminobutyric acid (GABA), herbicide-tolerant pigeon pea and flax, and pennycress and camelina with modified oil content.

The R & D pipeline (117 advanced-stage products and 292 early-stage products) shows a very diverse spectrum of applications in terms of traits and plants. New traits not previously obtained using established genomic techniques emerged. Disease resistance traits target more types of pathogens and pests. Abiotic stress tolerance has been widely explored, including in relation to resistance to drought, salinity and heat. Modified composition goes beyond starch and oil content, and many crops are emerging with better nutritional properties (fibre or vitamin content) or reduced content of potentially harmful compounds (toxins, allergens, acrylamide, etc.) or gluten. Furthermore, several applications are dedicated to obtaining higher and/or more stable yield (in terms of plant production and/or size of fruits or grains).

Among the various NGTs available, those based on CRISPR are preferred in most applications, as shown in **Figure 3**. CRISPR and other SDN techniques (based on TALEN, ZFN or meganucleases) are, in most cases, used to create mutations (small deletions/insertions) in the targeted site.

Figure 3. NGTs used in the plants identified in the database.



NB: 'Genome editing' refers to products for which no further specifics were provided.

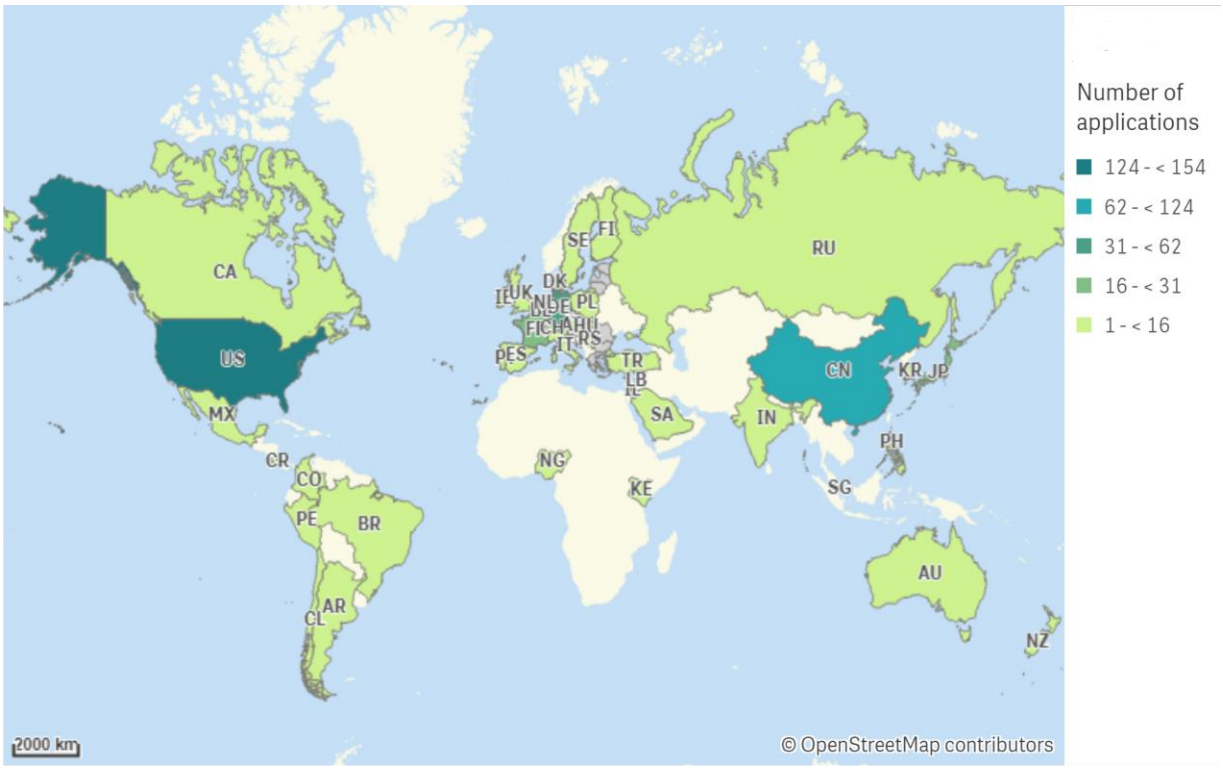
Source: Authors' research.

Regarding the country of origin of NGT-derived plants (i.e. the country of affiliation of the developers), **Figure 4** shows widespread use of these techniques, with a prevalence of US and Chinese applications. In the European Union countries (**Figure 4b**), the highest number of NGT applications come from Germany, followed by France. NGTs for plant production also show a high level of adoption in developing countries, as illustrated in **Box 1**.

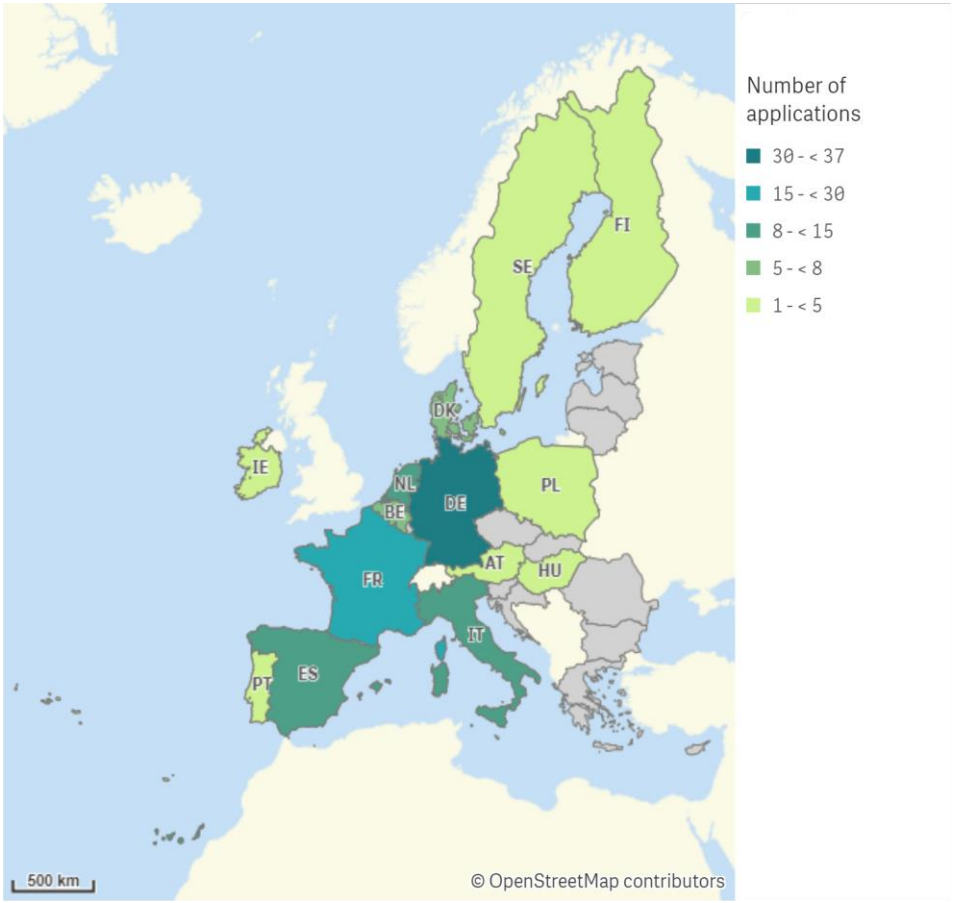
[4] Please, note that, after the finalisation of this report, we learned that one pre-commercial plant application (CRISPR/Cas-derived GABA tomato) moved further towards commercialisation and will soon be cultivated in Japan.

Figure 4. Geographical distribution of NGT developers of plants worldwide (**4a**) and in the EU (**4b**)

4a



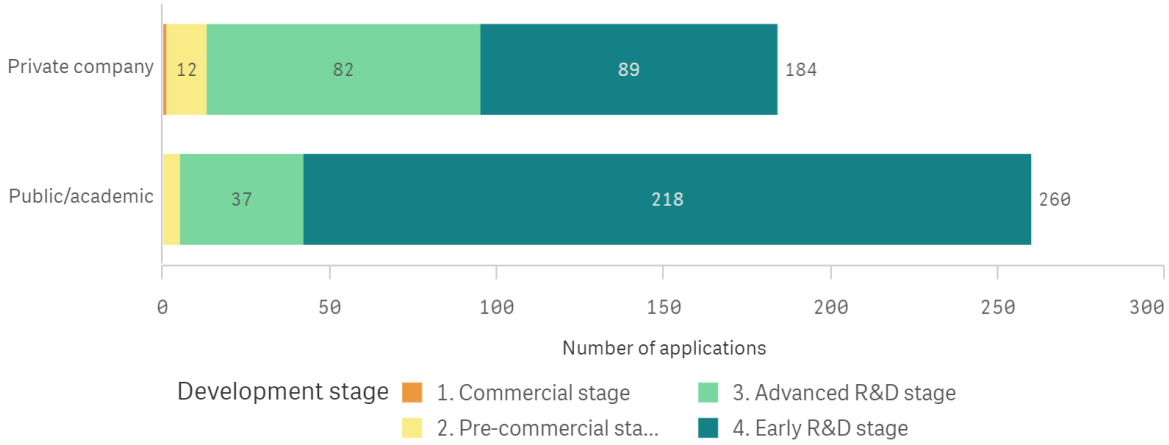
4b



Source: Authors' research.

Figure 5 shows the distribution of developers (whether private companies or public/academic developers, the latter including non-profit organisations). Private companies are more present in the near-market stages, while public/academic organisations dominate the early R & D results. This result could also be related to the sources of information used. Scientific literature is mostly produced by academia, while private companies are generally reluctant to disclose early information, as they wish to protect their business and intellectual property.

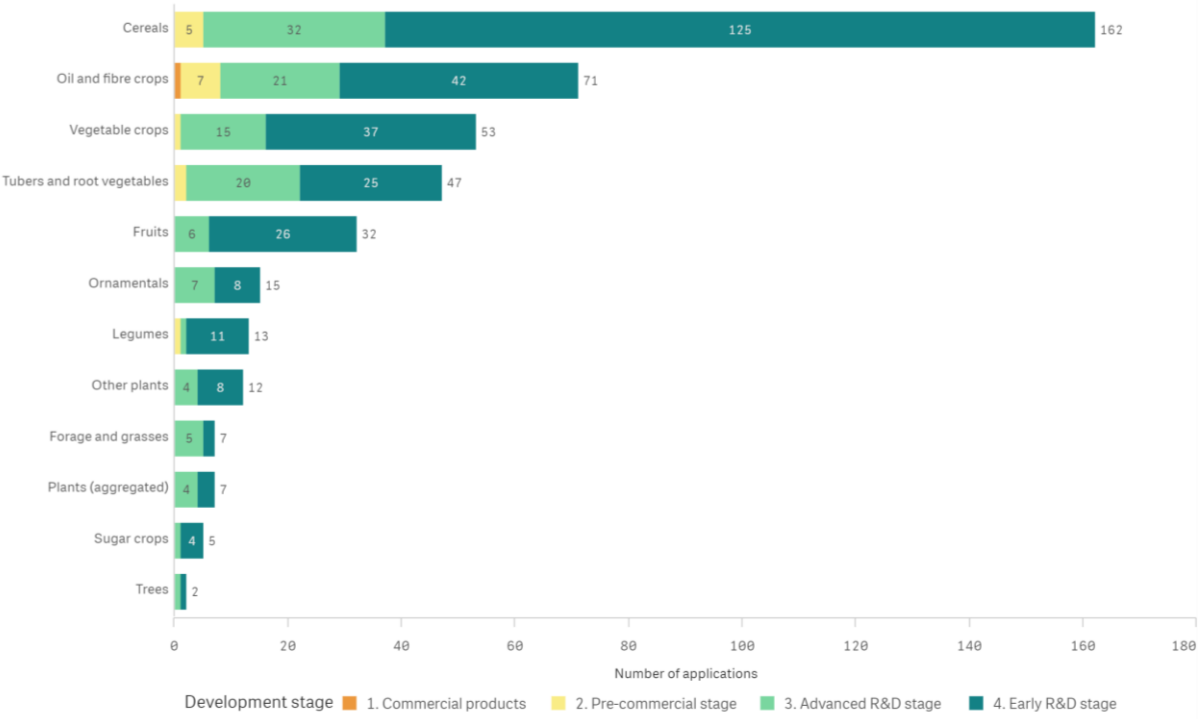
Figure 5. Distribution of NGT-produced plants by type of developer (private or public/academic) and development stage



Source: Authors' research.

Figure 6 shows the distribution of the 426 NGT-produced plants identified in our database by commercial development stage. NGTs are used mostly in cereals (162 products), followed by oil and fibre crops (71), vegetable crops (53), and tubers and root vegetables (47).

Figure 6. NGT-produced plants identified in the four development stages (commercial, pre-commercial, advanced R & D and early R & D), by plant group

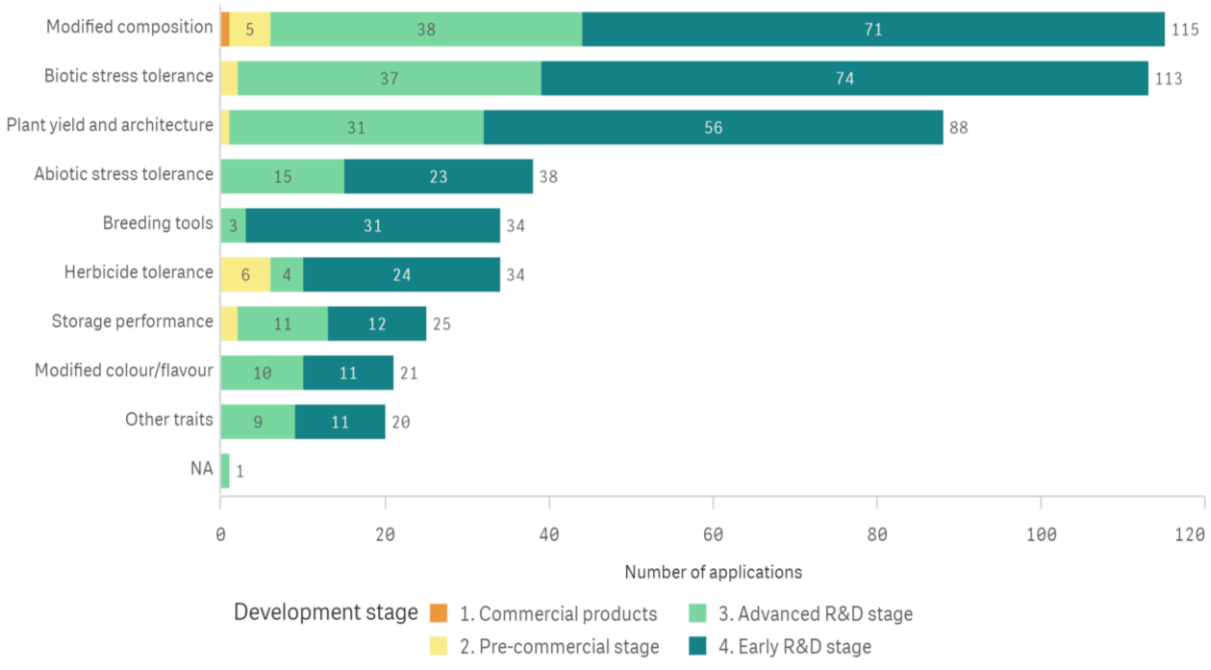


Source: Authors' research.

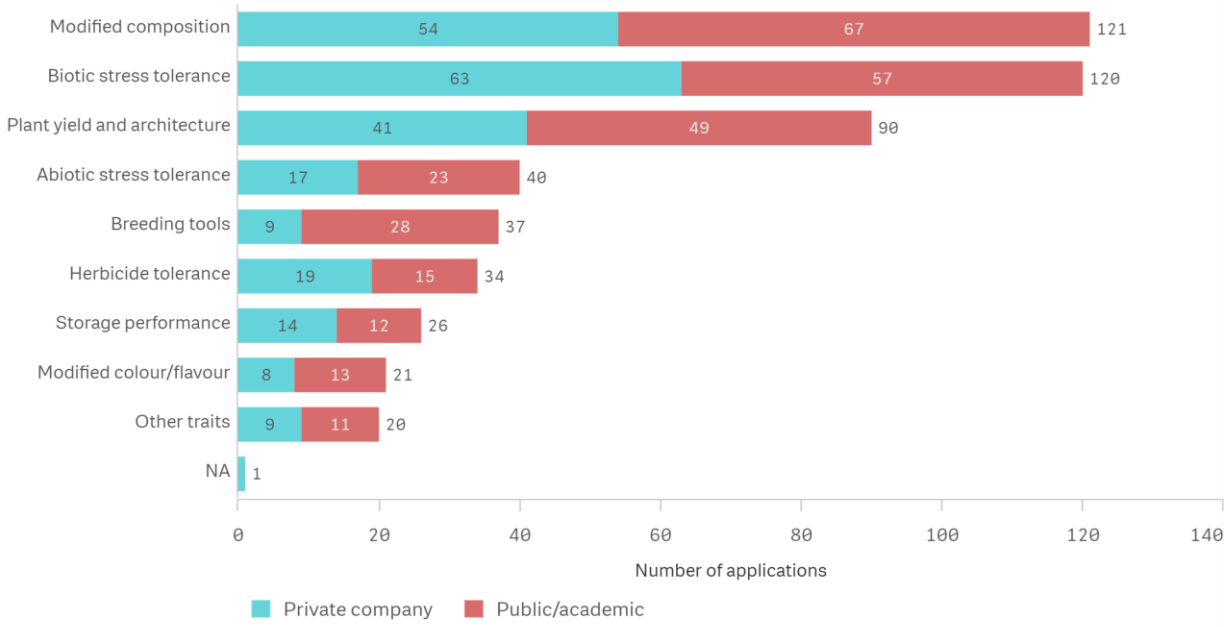
Figure 7 shows the NGT-produced plants by trait category and development stage (**Figure 7a**) and by trait category and type of developer (**Figure 7b**). Overall, biotic stress tolerance, modified composition and plant yield and architecture are the main goals in the use of NGTs in plants. Modified composition and herbicide tolerance are the most relevant at the commercial and pre-commercial stages. Private and public/academic developers seem to have a similar focus in terms of trait categories.

Figure 7. Entries in the database for NGT-derived plants by trait category and development stage (**7a**) and by trait category and type of developer (**7b**).

7a. Trait category and development stage



7b. Trait category and type of developer



NB: Note that some NGT plant entries are counted more than once in the figures because they have been edited for multiple traits and/or by multiple developers (this is also the reason why the total number of applications in each column is not the same in the two figures)

Source: Authors' research.

In addition to their use to obtain useful traits in the final plant variety, NGTs are also widely used as plant breeding tools. According to the information collected, in particular from technology providers, NGTs are used, among other purposes:

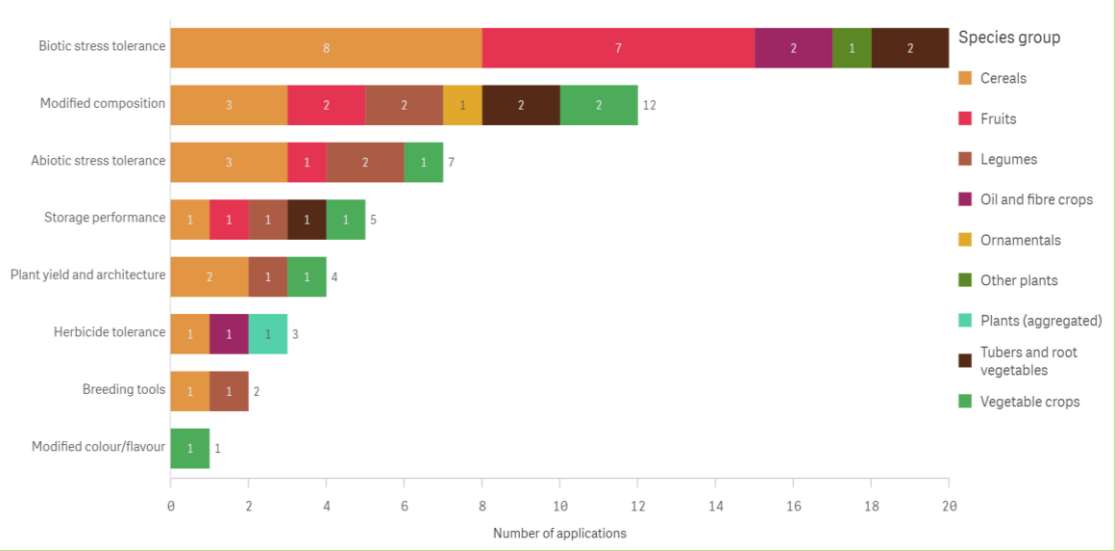
- to modify reproductive characteristics such as sterility, fertility restoration, self-compatibility or apomixes;
- to modify flowering characteristics such as early flowering;
- to increase or suppress genetic recombination;
- for haploid and double haploid induction.

Box 1. Plants produced using new genomic techniques in developing countries

Thanks to the flexibility and affordability of NGTs (especially the ones based on CRISPR), several developing countries are active in the field. In our data collection, China emerged as one of the most advanced countries, with 86 NGT products. In addition to those, 50 NGT-produced plant products were identified as being created in a developing country or by a consortium including organisations from developing countries* (see footnote at the end of the box). Those applications include one pre-commercial product in Brazil and 49 products still at the R & D stages (7 at the advanced stage and 42 at the early stage); considering that CRISPR/Cas has been discovered as a breeding tool very recently, this shows how rapidly it has reached plant breeding for developing countries' crops.

Figure 8 shows the characteristics of the abovementioned 50 plant products targeting developing countries in terms of trait and plant categories. According to the data collected, the main concern in developing countries is finding solutions against biotic stressors, followed by improving the nutritional properties of food (e.g. vitamin content) and increasing abiotic stress tolerance (especially with regard to drought and salinity).

Figure 8: NGT-derived plants from developing countries (excluding China), by trait category and plant group.



NB: Note that in some applications NGTs are used for multiple traits and are counted more than once in this figure

Source: Authors' research.

Of particular interest is the work done by CGIAR, a union of 15 independent, non-profit research centres conducting innovative research aimed at helping the world's poor. Eight of those centres have already incorporated NGTs, mostly CRISPR/Cas9, into their research projects, as shown in **Table 3**.

Box 1. NGT-produced plants in developing countries (continued)

Table 3: NGT-produced plant products developed by CGIAR

Research centre	Headquarters	Species	Trait category	SDN type (1, 2, 3)	Development stage
IITA	Ibadan, Nigeria	Banana	Biotic stress tolerance	SDN1	4. Early R & D stage
IITA	Ibadan, Nigeria	Banana	Biotic stress tolerance	SDN1	4. Early R & D stage
IITA	Ibadan, Nigeria	Banana	Biotic stress tolerance	SDN1	4. Early R & D stage
CIAT	Cali, Colombia	Beans	Modified composition	SDN1, SDN2	4. Early R & D stage
CIAT	Cali, Colombia	Cacao	Modified composition	SDN1, SDN2	4. Early R & D stage
CIAT	Cali, Colombia	Cassava	Haploid techniques	SDN1, SDN2	4. Early R & D stage
ICARDA	Beirut, Lebanon	Chickpea	Abiotic stress tolerance	SDN1	4. Early R & D stage
ICRISAT	Patancheruvu, India	Chickpea	Modified composition; plant yield and architecture	SDN1, SDN2	4. Early R & D stage
CIMMYT	El Batán, Texcoco, Mexico	Maize	Biotic stress tolerance	SDN1, SDN2	3. Advanced R & D stage
CIMMYT	El Batán, Texcoco, Mexico	Maize	Biotic stress tolerance	SDN1	3. Advanced R & D stage
ICRISAT	Patancheruvu, India	Millet	Storage performance	SDN1, SDN2	4. Early R & D stage
ICRISAT	Patancheruvu, India	Pigeon pea	Reproductive/flowering characteristics	SDN1, SDN2	4. Early R & D stage
CIP	Lima, Peru	Potato	Biotic stress tolerance	SDN1, SDN2	4. Early R & D stage
CIAT	Cali, Colombia	Rice	Biotic stress tolerance	SDN1, SDN2	3. Advanced R & D stage
CIAT	Cali, Colombia	Rice	Plant yield and architecture	SDN1, SDN2	4. Early R & D stage
IRRI	Los Baños, Philippines	Rice	Plant yield and architecture	SDN1	4. Early R & D stage
IRRI	Los Baños, Philippines	Rice	Modified composition	SDN1, SDN2, SDN3	4. Early R & D stage
IRRI	Los Baños, Philippines	Rice	Reproductive/flowering characteristics	SDN2	4. Early R & D stage
IRRI	Los Baños, Philippines	Rice	Biotic stress tolerance	SDN1	4. Early R & D stage
ICRISAT	Patancheruvu, India	Sorghum	Biotic stress tolerance	SDN1, SDN2	4. Early R & D stage
CIMMYT	El Batán, Texcoco, Mexico	Wheat	Biotic stress tolerance	SDN1	4. Early R & D stage
CIMMYT	El Batán, Texcoco, Mexico	Wheat	Biotic stress tolerance	SDN1	4. Early R & D stage

NB: CIAT, International Center for Tropical Agriculture; CIMMYT, International Maize and Wheat Improvement Center; CIP, International Potato Center; ICARDA, International Center for Agricultural Research in the Dry Areas; ICRISAT, International Crops Research Institute for the Semi-Arid Tropics; IITA, International Institute of Tropical Agriculture; IRRI, International Rice Research Institute.

Source: Authors' research.

* For the purpose of this analysis, developing countries are defined according to the list provided by the United Nations (United Nations, 2020).

3.1.1. Data gaps in plants

Several sources and experts were consulted, so the overall results can be considered to be representative of the current situation. Several attempts were made to identify and contact Chinese experts and validate the data collected, but unfortunately with no success. According to the experts consulted, there is no significant use of NGTs in the Chinese private sector, but the public sector is probably relevant, and more applications may be being developed than are included in our data; in other words, the pre-commercial pipeline may be even richer than our data collection indicates.

3.2. Mushrooms

For mushrooms, only one NGT application was identified: in a white button mushroom (*Agaricus bisporus*) developed by the University of Pennsylvania, which used CRISPR/Cas9 to delete the gene encoding polyphenol oxidase and thus obtain non-browning properties. According to the information available, these mushrooms were approved for commercialisation in the United States in 2016 and can be sold without further oversight (Waltz, 2016). However, no information about the actual commercialisation of this product was obtained. Therefore, it is considered to be at the pre-commercial stage.

3.3. Animals

According to the information collected, the pipeline in the animal sector contains fewer NGT applications than that in the plant sector. NGTs are mainly used for medical research purposes in model animals such as mice, monkeys, pigs and rats, and for food purposes, in particular in farm animals such as cattle, pigs and chicken, and in various fish (salmon, tilapia, tuna and red sea bream). Insects (especially mosquitos) and some invasive species are the subjects of NGT-based gene drive technologies. A summary of the animals to which NGTs are applied that are included in the database is provided in **Table 4**.

Animals to which NGTs are applied were classified in different groups according to the trait obtained / the goal of the technique, as shown in **Table 5**.

Since animals used as disease models are used exclusively for research and will never be put on the market, the findings related to this category are analysed separately in **Box 3**.

Table 4. Summary of animals identified in the database of NGT products

Organism	Species
Aquatic animals	Salmon, tilapia, tuna, carp, red sea bream, fugu, coral
Domestic animals	Cattle, pig, chicken, sheep, horse, dog
Rodents and primates	Mouse, rat, monkey
Insects	Mosquito, fly, moth
Other animals	Cane toad, feral cat

Source: Authors' research.

Table 5. Trait categories used for NGT animal products in the database

Trait category	Description
Biotic stress tolerance	Resistance to biotic stressors such as bacteria, viruses and other pathogens
Improved meat yield/quality	Including higher and faster meat production, modification in meat quality and muscle-related performance
Abiotic stress tolerance	Resistance to abiotic stressors such as high or low temperature
Hypoallergenic properties	Hypoallergenic properties of food derived from animals
Reproductive characteristics	Including changes in sexual characteristics such as sterility or the ratio of male to female offspring
Other traits	Traits not classified in the above categories, including management of herds, reduced toxin levels and modification of behavioural characteristics
Gene drive	Gene drive technology to pass a genetic modification to offspring, usually to eliminate pathogen-carrying insects or to control invasive species
Human therapy applications (described in Box 3)	Animals used as models to investigate gene therapies for human diseases (especially cancer and genetic diseases) or used to produce organs that can be used in human transplants

Source: Authors' research.

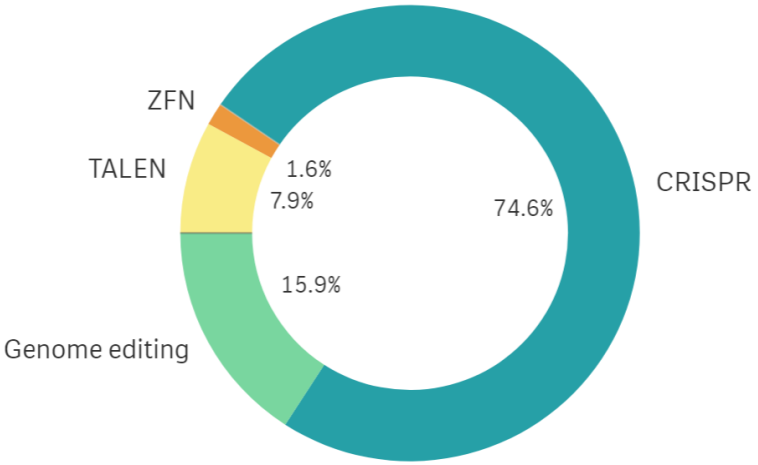
No NGT animals have yet been commercialised, while the pre-commercial pipeline includes four animals, all developed by private companies: Intrexon–AquaBounty yield-enhanced/fast-growing tilapia, Genus pigs resistant to porcine reproductive and respiratory syndrome in the United Kingdom, and, in the United States, Acceligen–Recombinetics hornless cattle (also reported in information from Australia and Brazil) and heat-resistant cattle (also reported in information from Argentina, Australia and Brazil).

The advanced R & D pipeline includes a total of 28 identified NGT applications, including 14 in domestic animals (6 in cattle, 5 in pigs, 2 in chickens and 1 in sheep), which, together with four fish products (red sea bream, salmon, tuna and carp), shows a predominance of food applications. Gene drive applications follow, with 7 applications in insects (5 in mosquitos, 1 in moths and 1 in flies) and 1 in mice.

The early R & D stage (31 applications identified in total) shows again a predominance of food-related applications in domestic animals and gene drive applications in insects, rats and feral cats. Some new traits emerged, such as modification of the muscle development of dogs and horses for better physical performance (e.g. greater strength or running ability).

Among the various NGTs available, CRISPR is the preferred platform in most applications, as shown in **Figure 9**. CRISPR and other SDN techniques (based on TALEN or ZFN) are, in most cases, used to create mutations (small deletions/insertions) in the targeted site. CRISPR is also used for gene drive, especially in insects, as explained in the following paragraphs.

Figure 9. NGTs used in the animal applications identified in the database.

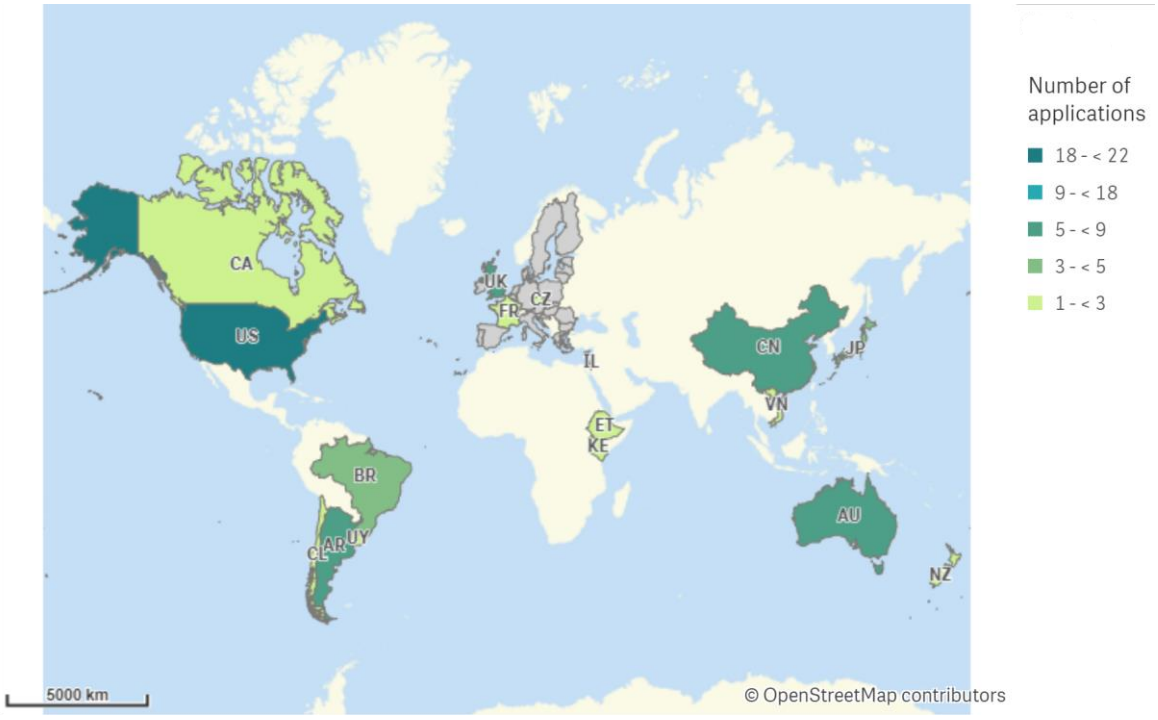


NB: 'Genome editing' refers to products for which no further specifics were provided.

Source: Authors' research.

Regarding the country of origin of these applications of NGTs in animals (i.e. the country of affiliation of the developers), **Figure 10** shows widespread use of these techniques, with a prevalence of US applications.

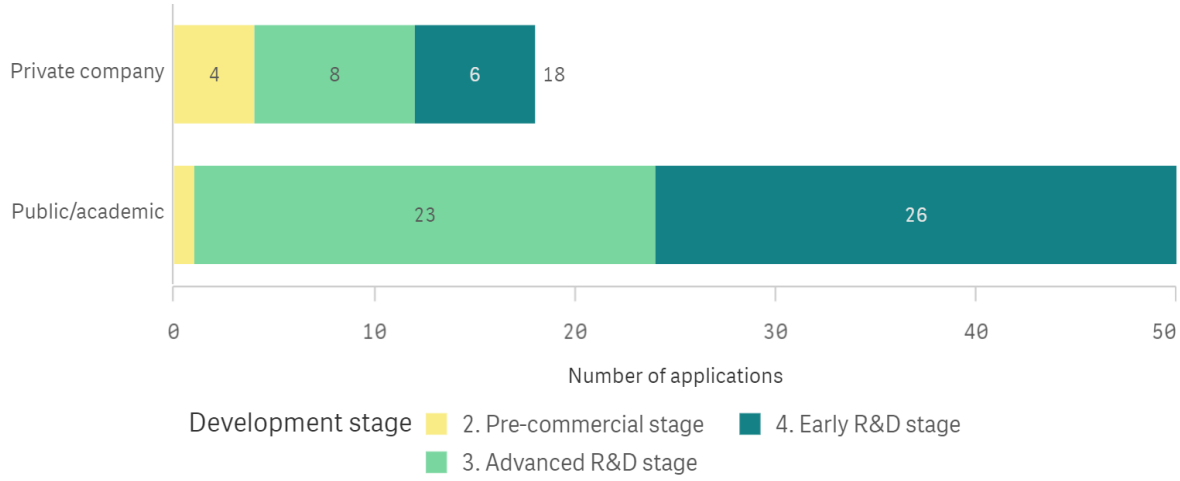
Figure 10. Geographical distribution of developers of NGT applications in animals worldwide



Source: Authors' research.

Figure 11 shows the distribution of developers (whether private companies or public/academic developers). Private companies have developed a higher number of pre-commercial applications, while many advanced- and early-stage R & D applications have been developed in the public/academic sector. Again, this result could also be related to the sources of information used, as explained in **Section 3.1** on plants.

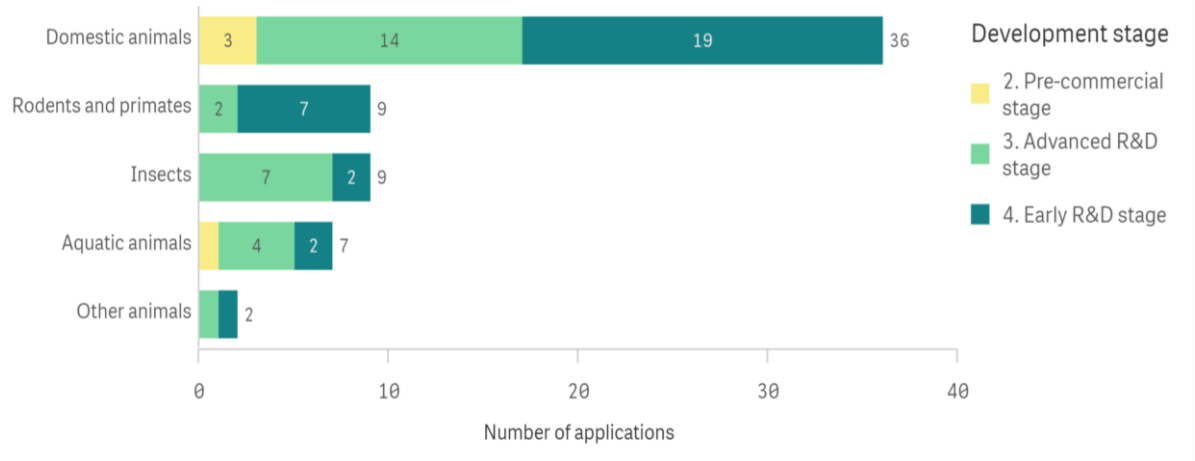
Figure 11. Distribution of NGT applications in animals by type of developer (private or public/academic) and development stage



Source: Authors' research.

Figure 12 shows the distribution of the 63 applications of NGTs in animals identified in our database by commercial development stage. NGTs are used mostly in domestic animals (36 entries).

Figure 12. Applications of NGTs in animals identified in the pre-commercial, advanced R & D and early R & D stages (no animals at the commercial stage were identified), by animal group

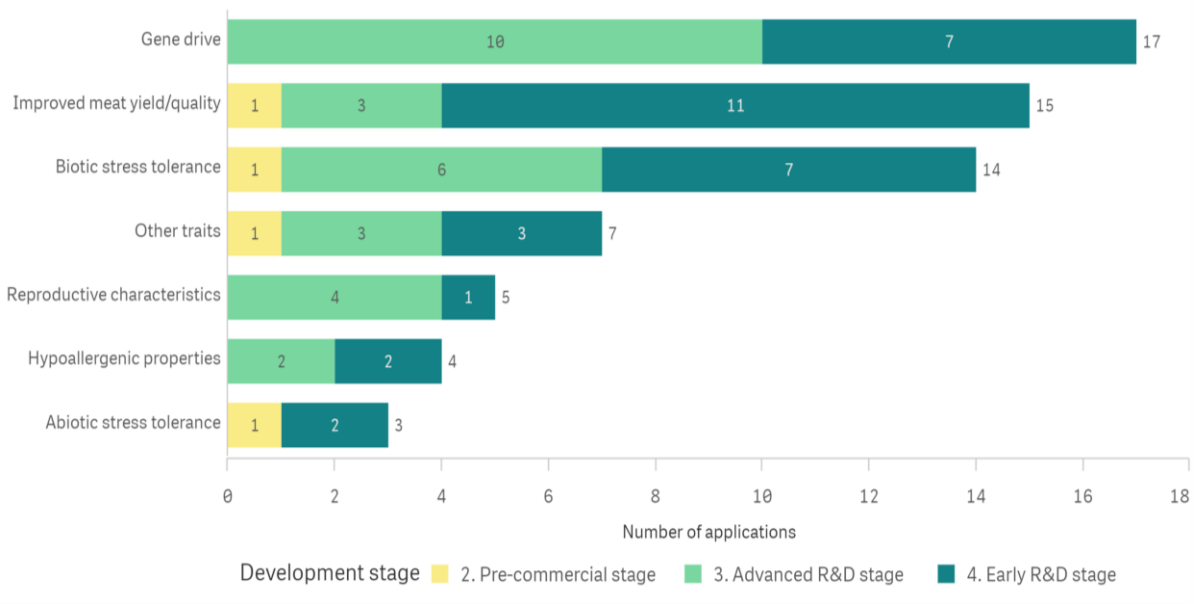


Source: Authors' research.

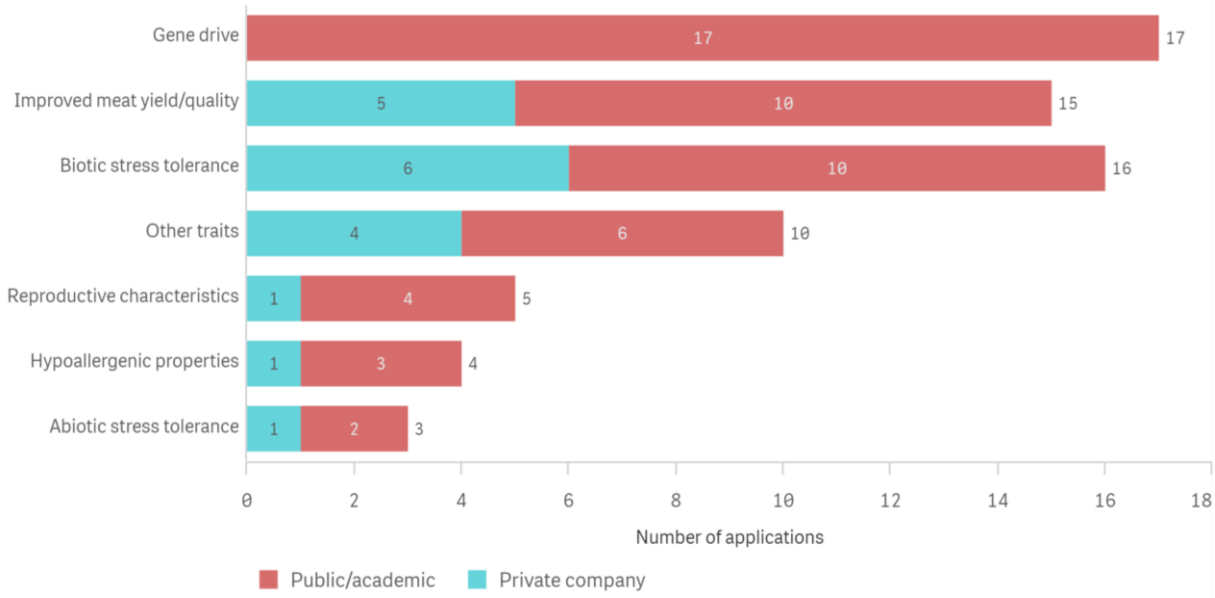
Figure 13 shows the applications of NGTs in animals by trait category and development stage (**Figure 13a**) and trait category and type of developer (**Figure 13b**). The highest number of applications are for gene drive purposes (17), improved meat yield/quality (15) and biotic stress tolerance (14), the last relating to animal diseases such as porcine reproductive and respiratory syndrome.

Figure 13. Entries in the database for NGT applications in animals by trait category and development stage (**13a**) and by trait category and type of developer (**13b**).

13a. Trait category and development stage



13b. Trait category and type of developer



NB: Note that some NGT animals are counted more than once in the figures because they have been edited for multiple traits and/or by multiple developers (this is also the reason why the total number of applications in each column is not the same in the two figures)

Source: Authors' research.

The gene drive animals identified are obtained using CRISPR and mostly with the aim of stopping the spread of mosquito-driven diseases (e.g. malaria and dengue) or of invasive species (mice, rats, carp and feral cats), that is, non-native species that cause ecosystem disruption in specific geographical areas. In the latter case, the gene drive system is based on spreading the daughterless trait.

The NGT uses identified confirm that there is a significant focus on breeding animals (especially farm animals) for disease resistance and reproductive characteristics (e.g. sterility in farmed fish to avoid

crossbreeding with wild species), and there is also a strong focus on meat yield and quality, through modification of muscle content.

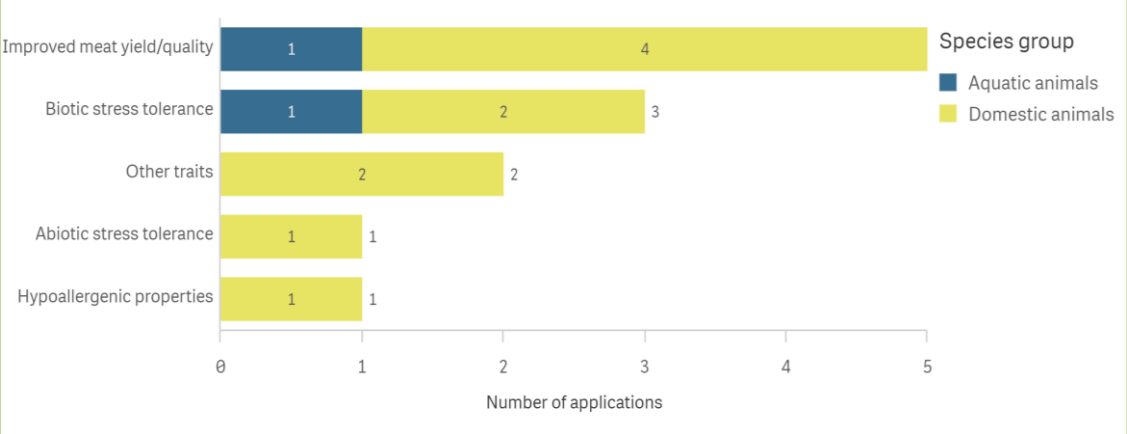
Gene drive research is conducted only by public/academic developers, while the other trait categories are sought by both types, with a slight predominance of public/academic developers.

Box 2. New genomic techniques in animal breeding in developing countries

Animal breeding using NGTs is also a reality in developing countries, although there are fewer animal applications than plant applications. In our data collection, 12 NGT animal applications were identified as having been created in a developing country (excluding China) or by a consortium including developing countries, mainly in Argentina (six applications) and Brazil (five applications), including, between those two countries, three pre-commercial applications.

Figure 14 shows the characteristics of the abovementioned 12 NGT animal applications targeting developing countries in terms of trait and animal categories. According to the data collected, the main concern in developing countries is improved meat yield/quality, followed by increased biotic stress tolerance.

Figure 14: NGT animal applications from developing countries (excluding China), by trait category and animal group.



NB: Note that in some applications NGTs are used for multiple traits and are counted more than once in this figure.

Source: Authors' research.

As explained in **Box 1**, CGIAR plays an important role in bringing innovative tools to developing countries. For completeness, the information received about one NGT animal product by CGIAR is reported in **Table 6**.

Table 6. NGT animal products developed by CGIAR.

Research Centre	Technique	SDN type (1, 2, 3)	Species	Trait category	Development stage
ILRI	Gene editing	SDN3	Cattle	Biotic stress tolerance	4. Early R & D stage

NB: ILRI, International Livestock Research Institute

Source: Authors' research.

Box 3. Animal models for human diseases

One particular use of NGTs in animals is in the field of research on human diseases, using animals as disease models to search for a gene therapy or using animals to produce organs that can be transplanted into human patients.

Table 7 shows the NGT applications identified in this field. As stated in the methodology section, the use of NGTs to cause disease in animals is excluded from the scope of this study, while the use of NGTs to cure disease or to help in creating human-compatible organs is included, since these applications have the potential to reach the patients. The list provided in **Table 7** is not comprehensive but meant to provide a representative description of the sector.

According to the information collected so far, mice are the model animals in which NGTs are most commonly applied in human gene therapy studies (in particular in studies on cancer and genetic diseases). Pigs will be particularly important for the future production of organs that do not cause transplant rejection in humans. NGT-modified rats and monkeys are also used to model human diseases, but this application is still at the early R & D stage.

Animals used as human disease models are the subjects of four applications identified as being at the advanced R & D stage: three applications in mice to find a cure for human health conditions (prostate cancer, traumatic encephalopathy and human immunodeficiency virus (HIV) infection) and one in pigs to produce compatible organs. The early R & D stage shows a greater variety of applications in terms of diseases and animals.

Table 7. NGT-edited animals as human disease models

Development stage	Species group	Species	Number of products	Trait description/Condition targeted
3. Advanced R & D stage	Domestic animals	Pig	1	Traumatic shock or liver failure
3. Advanced R & D stage	Rodents and primates	Mouse	3	Chronic traumatic encephalopathy, prostate cancer, HIV
4. Early R & D stage	Domestic animals	Pig	3	Organ compatibility
4. Early R & D stage	Rodents and primates	Monkey	2	Organ compatibility, infections including HIV infection
4. Early R & D stage	Rodents and primates	Mouse	24	Muscular dystrophy including Duchenne muscular dystrophy, haemophilia B, Huntington's disease, Leber congenital amaurosis, retinitis pigmentosa, Meesmann epithelial corneal dystrophy, open-angle glaucoma, acute kidney disease, cancer including leukaemia, cardiovascular disease, high cholesterol, hyperlipidaemia, obesity, type II diabetes, heart failure and renal failure, human papillomavirus, pancreas compatibility
4. Early R & D stage	Rodents and primates	Rat	3	Pancreas compatibility, retinitis pigmentosa

Source: Authors' research.

3.3.1. Data gaps in animals

Fewer animals produced using NGTs have been identified than NGT-derived plant products. This most probably reflects the reality, but the number of experts who contributed information on the plant sector to the database was much higher than that for the animal sector. Furthermore, as in the case of plants, no experts from the Chinese public sector were reached who could validate and integrate the data.

3.4. Microorganisms

Microorganisms have a wide range of uses in the industrial sector, either in contained use to produce specific molecules (that constitute the final commercial product) or for deliberate release as living organisms, as summarised in **Table 8**.

Table 8. Examples of uses of microorganisms in industrial biotechnology

Use of microorganisms	Contained use	Deliberate release
Food/feed-related applications	Product ion of: • Food enzymes (for baking, starch products, plant-based proteins, vegetable oil, dairy products, meat processing) • Feed enzymes (to increase nutritional value) • Food/feed ingredients	• Probiotics for animal and human health • Microorganisms in feed (to improve nutrition or feed conversion ratio) • Inoculants (substitutes for fertilisers) • Biocontrol (substitutes for pesticides)
Non-food/feed	• Enzymes (for use in detergents, textiles, leather, pulp and paper) • Biofuels • Cosmetics • Pharmaceuticals • Other bio-based building block chemicals	• Bioremediation

Source: Authors' research.

According to the experts consulted, NGTs have potential in microorganism strain improvement and are already being used by several companies. They are becoming standard tools, along with established techniques such as classical mutagenesis, homologous recombination and self-cloning.

The experts stated that intellectual property rules and regulatory frameworks, as well as product life cycles and marketing decisions, would determine their entry into the global market. Typically, there are more safety requirements worldwide relating to deliberate release of microorganisms than relating to contained use (where the microorganism is used as bio-factory and not as a final commercial product), and for food/feed than for other industrial applications. Therefore, it appears that the uptake of NGTs to improve microorganism strains may be faster in contained use and especially in the bioeconomy sector, for example to produce biofuels or bio-based (non-food/feed) chemicals.

3.4.1. New genomic techniques applied to microorganisms that constitute the final commercial product (deliberate release)

One market sector is the commercialisation of microorganisms as final products for their release into the environment. Here, we identified one commercial NGT application in nitrogen-producing soil bacteria able to fertilise cereal crops and thus used as supplement to nitrogen fertilisers. This product, called Pivot Bio PROVEN, developed by the US company Pivot Bio, is already commercialised in the United States.

Another example, at advanced R & D level, is CRISPR-edited *Epichloë coenophiala* (an endophyte fungus of tall fescue, a type of pasture grass) to eliminate production of compounds (ergot alkaloids) that are toxic to livestock (University of Kentucky). The R & D pipeline includes other examples of applications in soil bacteria and in probiotics (data not disclosed for confidentiality reasons).

3.4.2. Use of new genomic techniques to improve microorganism strains for producing industrial molecules (contained use)

In line with what was stated above, from the online information search and the input from private companies, we identified applications of NGTs in microorganisms for the contained production of molecules of industrial interest (e.g. biofuels and enzymes) that are already on the market in at least one country worldwide (data not disclosed for confidentiality reasons).

The R & D pipeline for applications of NGTs in microorganisms for contained use is also rich at pre-commercial and R & D levels. Some examples are provided below.

Pre-commercial stage.

- CRISPR-edited microorganisms developed by the US company Solugen produce an enzyme for hydrogen peroxide production from plant sugar.
- Genome-edited *Saccharomyces cerevisiae* (four different lineages for bioethanol production) has been approved by the Brazilian National Technical Commission on Biosafety (CTNBio) as non-GMO
- The genome-edited product BiomElix Guided Biotic, *Escherichia coli* for control of Salmonella infection in broiler birds, has been added to wastewater and approved by CTNBio as non-GMO

Advanced R & D stage.

- CRISPR-edited microalgae (*Nannochloropsis*) for biodiesel production have been developed by the US companies Synthetic Genomics and ExxonMobil.
- CRISPR-edited yeast for ethanol production has been developed by GlobalYeast JV Co Brasil.
- CRISPR-edited *E. coli* for the production of biodiesel has been developed by Washington University.

The use of NGTs in microorganisms requires a particular approach. In plants and animals, we identified NGT applications generally in organisms in which no other genetic modification or gene editing techniques had already been applied. In microorganisms for industrial uses, technology developers usually apply a full set of techniques (including both established and new genomic techniques) to modify a specific strain until they reach the desired goal.

Companies tend to use a limited number of microorganism strains (usually bacteria, yeasts and fungi) that have a long history of safe use and that have already been optimised as bio-factories. Therefore, new properties are often added to strains that have been previously modified/edited for other commercial purposes.

Since microorganisms used at market or near-market level are usually the product of several (established and new) genomic techniques, it is difficult to provide a meaningful list of organism + NGT + trait combinations, as was done for plants and animals.

In microorganisms, the most common use of NGTs is to knock out unfavourable genes, encoding, for example, for toxins, intrinsic antibiotic resistance or by-products. Established techniques such as homologous recombination and classical mutagenesis allow companies to achieve all the desired changes, but NGTs can considerably reduce the time needed in certain cases, for example when multiple copies of the same gene need to be knocked out. Among NGTs, CRISPR is the favoured tool because of its speed, efficiency and low cost.

Box 4. New genomic techniques in microbiotic medicinal products

According to one of the experts consulted, NGTs (especially the ones based on CRISPR) are being adopted by many developers to edit the human microbiome for therapeutic purposes.

Two types of potential uses were described.

Microorganisms can be administered to patients, becoming part of the patient's microbiome and producing therapeutic molecules (e.g. interleukins). Several companies are active in this field and some of them are using CRISPR as a tool in the development of these microorganisms. No detailed information was identified online, but two of these companies mention CRISPR on their websites.

- The BioPlx, Inc. website (http://www.bioplx.com) makes general mention of CRISPR as a tool to edit the microbiome for cancer treatment.
- Novome Biotechnologies, Inc. (https://novomebio.com) has licensed CRISPR/Cas9 intellectual property controlled by Caribou to accelerate the development of pre-clinical candidates for chronic disease treatment (Powell, 2020).

A less advanced field is the potential use of microorganisms (phages or bacteria) as vectors to modify the microbiome *in vivo*, in the body of a patient (e.g. to knock out antibiotic resistance genes or virulence genes in pathogens).

Two companies have been identified using CRISPR in this field.

- Eligo Bioscience (https://eligo.bio/technology/) is researching the use of CRISPR in this field, but still at the early R & D stage. On its website, the company makes reference to two publications (Bikard et al., 2014; Citorik et al., 2014), describing results in model organisms (moth and mouse).
- Locus Biosciences (https://www.locus-bio.com/) was planning a clinical trial at the end of 2019 in the United States using bacteriophages to target antibiotic-resistant *E. coli* in the urinary tract (Stein, 2019).

3.4.3. Data gaps in microorganisms

Collecting data on NGT-derived microorganisms was challenging in terms of identifying data sources. The survey of private companies resulted in a relatively small number of companies participating, compared with those providing information on the plant sector, and generally the answers disclosed less detail (e.g. microorganism strain, specific technique used or specific trait obtained) because of confidentiality concerns. In particular, no sources of data on pharmaceutical and cosmetic products derived from NGT microorganisms were identified. Finally, the resulting data cannot be aggregated in a similar way to those for plants, animals and human cells, because of scarcity and heterogeneity. Therefore, no figures (and no visualisation in the web dashboard) are presented.

3.5. Human health

NGTs are now widely employed in medical/therapeutic applications. Their use in animals as human disease models is described in **Section 4.2**. This section is dedicated to NGTs in human cells, which includes their use (1) *in vitro*: the targeted human cells are treated in the lab (and never delivered to the patient); (2) *in vivo*: the NGT-based therapy is administered directly to the patient (i.e. the targeted cells remain always in the body of the patient); and (3) *ex vivo*: the targeted cells are removed from the patient and then returned after the NGT has been applied to the cells *in vitro*.

The main NGT applications for human health use are the following ([5]):

- Chimeric antigen receptor (CAR) T cells that recognise and destroy cancer cells are used in cancer immunotherapy. CAR T cells are modified with CRISPR/Cas9 for transgene insertion and knockdown of endogenous T cell genes in order to reduce graft-versus-host reactions (which opens up the possibility of developing allogeneic T cells to be used for several patients) ([6]).
- Treatment of viral infections: several methods are being explored of using NGTs to produce antiviral therapeutics, either by altering the host genes required by the virus or by targeting the viral genes necessary for replication (Chen et al., 2018a). For instance, CRISPR/Cas9-modified T cells that recognise viral antigens are being employed for treatment of infections with viruses such as HIV.
- Stem cell-based gene therapy, usually with induced pluripotent stem cells and haematopoietic stem and progenitor cells modified *in vitro* with NGTs, is usually employed to treat hereditary haematological diseases (e.g. sickle cell anaemia and β-thalassemia) and neurodegenerative diseases (e.g. Huntington's disease, Alzheimer's disease and Parkinson's disease), among other conditions.
- NGTs can be used for gene correction *in vivo* in the human body (e.g. of retinal photoreceptors in patients with Leber congenital amaurosis). *In vivo* correction of blood cells is also being researched for cardiovascular diseases.
- RNA editing can be carried out by treatment with antisense oligonucleotides recruiting endogenous adenosine deaminases acting on RNA (ADAR) enzymes to specific transcripts for oligonucleotide-mediated RNA editing (RESTORE technology) (Merkle et al., 2019).

Some NGT applications for medical/therapeutic purposes have gone beyond pre-clinical research and are reported in clinical trials, which are at either phase I or phase I/II (phases I and II are usually merged for rare diseases with small numbers of patients); that is, they have not yet reached a commercial or pre-commercial stage. A total of 63 NGT clinical trials have been identified in this study, of which 5 relate to diagnostic tests (covered in **Section 3.5.1**), 2 are long-term follow-up studies (i.e. patients who participated in a clinical trial are continuing the treatment to evaluate the long-term effects) and 5 are indicated as 'withdrawn'. Data from literature (61 scientific papers) are also reported and are intended to give a clear overview of the potential of NGTs; however, the list of identified papers is not exhaustive.

Applications in patients *in vivo* and *ex vivo*, which included most of the applications in clinical trials, were classified as at the advanced R & D stage. *In vitro* applications (described in most of the scientific papers) were classified as at the early R & D stage.

The 119 applications identified (plus 5 in diagnostic tools) relate to the conditions indicated in **Table 9**.

([5]) For an overview of NGT applications in the medical field, see Carroll (2016) and Li et al. (2020).
([6]) For a review of this type of NGT application, see Ren and Zhao (2017).

Table 9. Conditions targeted by NGT applications for human health.

Condition category	Conditions	No of literature sources	No of clinical trials			
			Total	Withdrawn	Long-term	Diagnostics
Cancer	Leukaemia, lymphoma, gastrointestinal cancers, lung cancer, bladder cancer, prostate cancer, pancreatic cancer, myeloma, melanoma, glioma, ovarian cancer, renal carcinoma, neurofibromatosis	20	30	5	—	2
Viral diseases	HIV-1, coronavirus disease 2019 (COVID-19), norovirus	13	11	—	—	1
Neurodegenerative diseases	Huntington's disease, Alzheimer's disease, Parkinson's disease	6	—	—	—	—
Hereditary haematological disease	β-Thalassemia, sickle cell disease, haemophilia B	6	10	—	2	
Hereditary eye diseases	Leber congenital amaurosis type 10	4	1	—	—	—
Virus-related cancer	Human papillomavirus-induced cancer, Epstein–Barr virus-positive cancer, HIV-1 infection with Acute lymphoblastic leukemia	3	5	—	—	—
Cardiovascular and metabolic diseases	PRKAG2 cardiac syndrome, hypertrophic cardiomyopathy, obesity, diabetes	4	—	—	—	—
Lung infection	Tuberculosis, severe sepsis	—	2	—	—	2
Other hereditary diseases	Mucopolysaccharidosis types I and II, Kabuki syndrome 1, Rubinstein–Taybi syndrome, deafness	5	4	—	—	—
Total		**61**	**63**	**5**	**2**	**5**

NB: PRKAG2: 5'-AMP-activated protein kinase subunit gamma-2.

Source: Authors' research.

Figures 15–19 do not show data on applications for diagnostic purposes, which are described in **Section 3.5.1**.

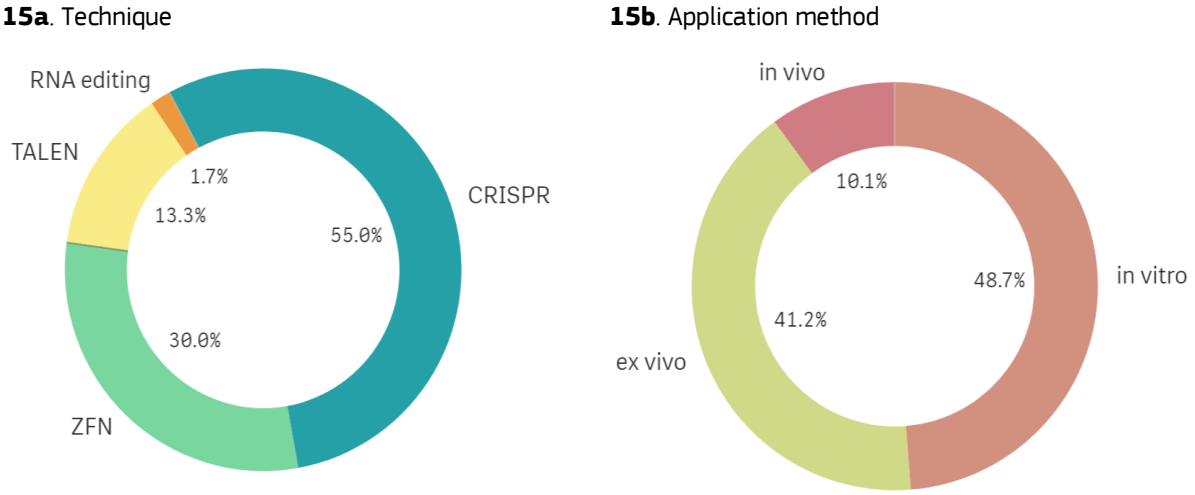
The NGT platforms used for medical/therapeutic applications focusing on human diseases are mainly ZFN, TALEN and CRISPR, as shown in **Figure 15a**, with CRISPR prevailing during the past 5 years.

Figure 15b shows that the applications identified use NGTs *in vitro* and *ex vivo* in similar numbers, with significantly fewer *in vivo* uses (about 10 %).

Regarding the countries of origin of the 119 NGT applications for human health ([7]), **Figure 16** shows that they are predominantly in the northern hemisphere (Australia is an exception). The United States and China, as in other sectors analysed, are the strongest countries, with the United States prevailing in terms of number of applications identified.

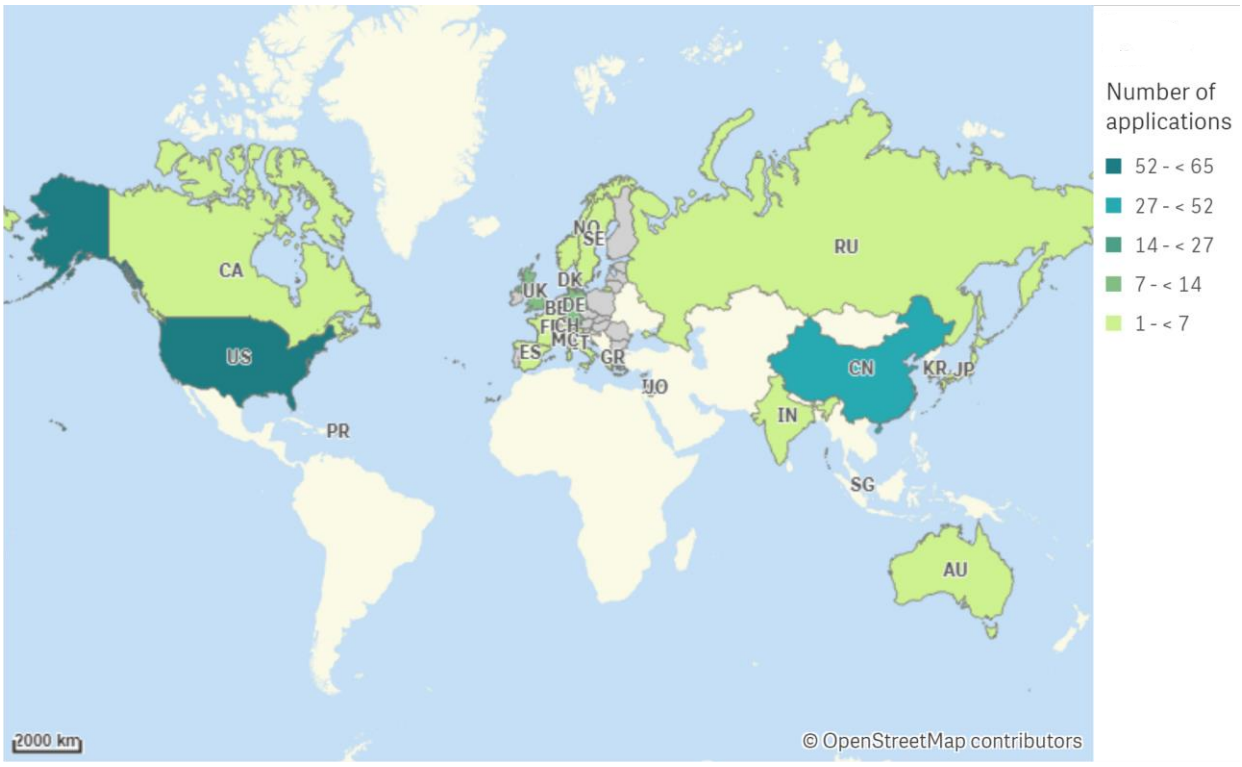
([7]) Countries correspond to author affiliations in the case of literature and to locations in the case of clinical trials

Figure 15. NGTs used for human health (*n* = 119) (**15a**) and their application methods (**15b**), according to the information in the database



Source: Authors' research.

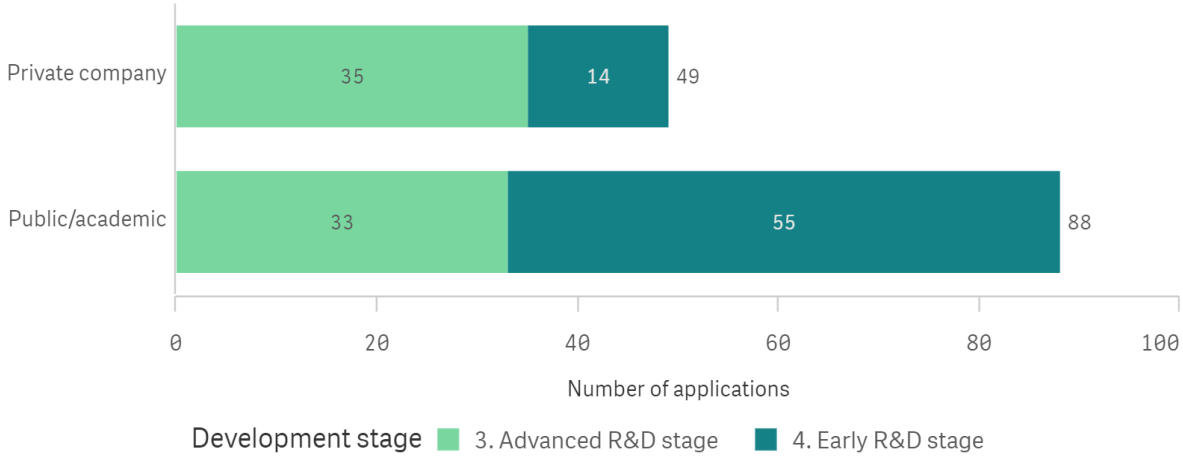
Figure 16. Geographical distribution of NGT applications for human health worldwide



Source: Authors' research.

As shown in **Figure 17**, a similar number of applications at the advanced R & D stage (i.e. the majority used in clinical trials) come from private and public/academic technology providers, while applications at the early R & D stage (i.e. most of those identified from literature data) are dominated, as in other sectors analysed, by public/academic authors.

Figure 17. Distribution of developers of NGT applications for human health by type of developer (private or public/academic) and development stage



NB: Developers' organisations correspond to author affiliations in the case of literature and to organisations carrying out the clinical trials (see footnote 5).

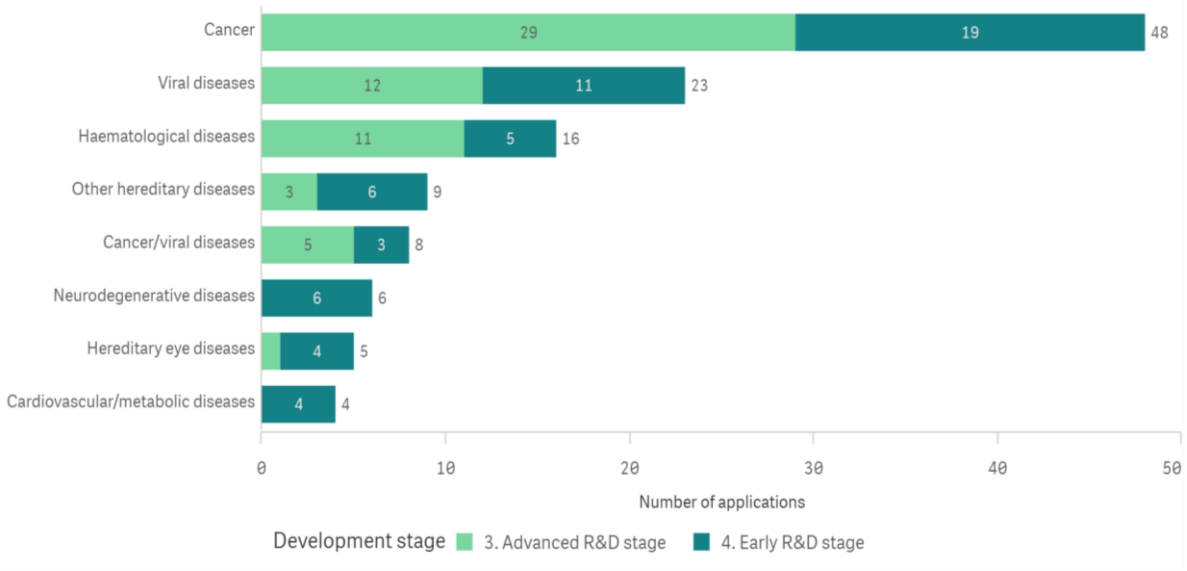
Source: Authors' research.

Figure 18 shows that cancer is clearly the main target of therapeutic applications involving the use of NGTs, with 48 applications, plus 8 applications relating to types of cancer caused by viral infections. The second most frequent target is viral diseases (23 applications), and many applications target hereditary diseases, including haematological diseases (16 applications), some neurodegenerative diseases (2 applications for Huntington's disease), eye diseases (5) and other hereditary diseases (8).

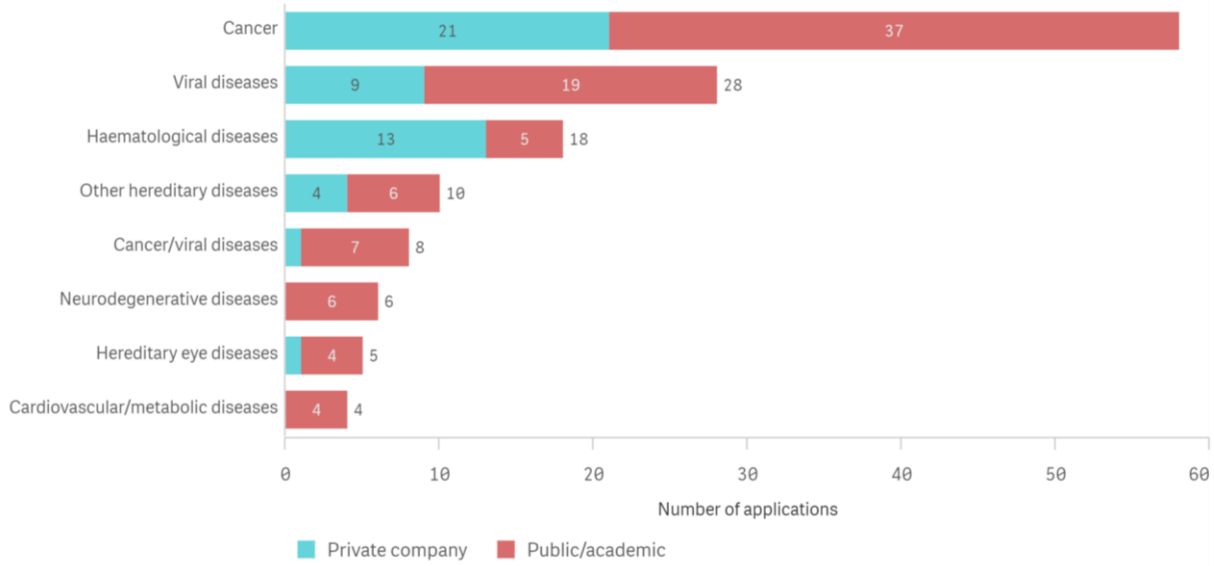
Figure 18b shows the prevalence of private developers in the treatment of haematological diseases, while public/academic developers prevail in all other condition groups.

Figure 18. Distribution of NGT human health applications by disease group and development stage of the therapy (**18a**) and by disease group and type of developer (**18b**).

18a. Disease group targeted and development stage



18b. Disease group targeted and type of developer

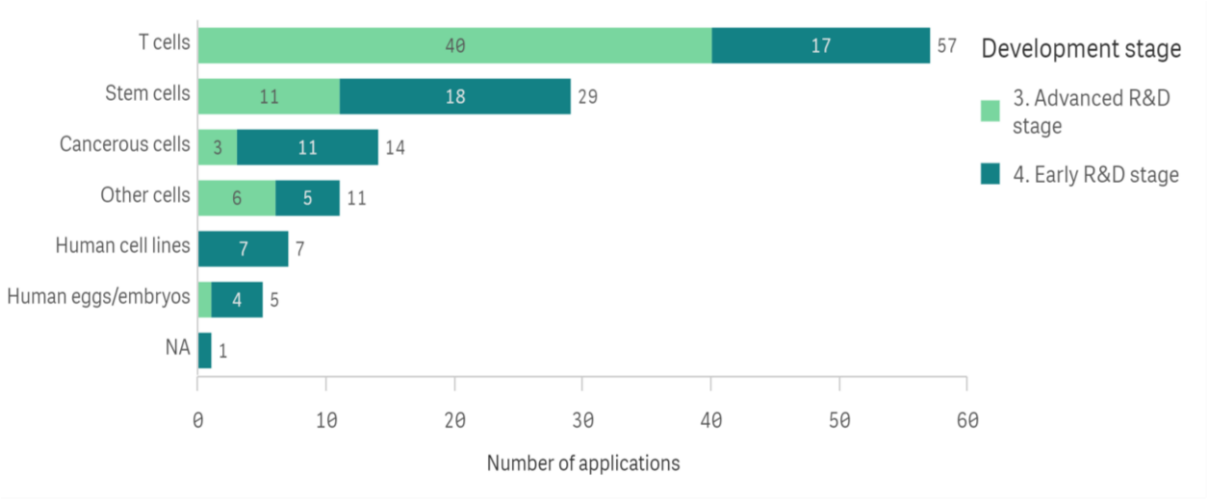


NB: Note that some applications are counted more than once in the second figure because they have multiple developers (this is also the reason why the total number of applications in each column is not the same in the two graphs)

Source: Authors' research.

Figure 19 shows the types of human cells to which NGTs are applied for therapeutic purposes. The cell type most frequently targeted is T cells (mostly autologous but in some cases allogenic), followed by stem cells and cancerous cells.

Figure 19. Distribution of NGT human health applications by target cell type and stage of development



Source: Authors' research.

3.5.1. Diagnostic tools based on new genomic techniques

Five clinical trials have been identified in which CRISPR/Cas is used as a diagnostic tool. **Table 10** provides a list of these trials. Three trials in China are focused on infectious diseases, and one of these targets COVID-19 diagnosis. The other two trials, in the United States, are focused on cancer diagnosis.

Table 10. Clinical trials in which NGTs are used for diagnostic purposes

NGT	Identifier	Status	Study title	Condition category	Conditions	Locations	Country	Sponsors and collaborators
CRISPR/Cas9	ChiCTR2000029810	Not approved, recruiting	Clinical study of a novel high sensitivity nucleic acid assay for novel coronavirus pneumonia (COVID-19) based on CRISPR-Cas protein	Viral disease	COVID-19	N/A	China	National Key R & D Program of China, High-level Hospital Construction Fund of Guangdong Province, Sanming Project of Medicine in Shenzhen
CRISPR/Cas12	NCT04178382	Recruiting	Effect of PCR-CRISPR/Cas12a on the early anti-infective schemes in patients with open air pneumonia	Lung infection	Severe sepsis	The Affiliated Drum Tower Hospital, Medical School of Nanjing University	China	Chinese Medical Association
CRISPR/Cas9	NCT04074369	Recruiting	Evaluation of CRISPR-based test for the rapid identification of tuberculosis in pulmonary tuberculosis suspects	Lung infection	Pulmonary tuberculosis	Huashan Hospital, Shanghai	China	Huashan Hospital, Wenzhou Central Hospital, Hangzhou Red Cross Hospital
CRISPR/Cas9	NCT03332030	Suspended (owing to cessation of funding)	Stem cells in neurofibromatosis type 1 patients with tumours of the central nervous system	Cancer	Neuro-fibromatosis type 1	Children's National Medical Centre, Washington	United States	Roger Packer
CRISPR/Cas9	NCT03606486	Recruiting	Lavage of the uterine cavity for diagnosis of ovarian cancer	Cancer	Ovarian cancer	Fred Hutch/University of Washington Cancer Consortium, Seattle	United States	University of Washington National Cancer Institute, Minnesota Ovarian Cancer Alliance

Source: Authors' research.

In addition, according to the information retrieved from literature and web sources, the following detection systems have been developed based on CRISPR and can be used for diagnostic purposes.

- **SHERLOCK** (Specific High-sensitivity Enzymatic Reporter unLOCKing) detects low-frequency mutations (e.g. those related to an increased risk of cancer, such as BRAF V600E and EGFR L858R). The system consists of two elements, the RNA-guided endonuclease Cas13a and the reporter signal, released after RNA cleavage (Gootenberg et al., 2017).
- **CARMEN** (Combinatorial Arrayed Reactions for Multiplexed Evaluation of Nucleic acids), is based on SHERLOCK and is a platform for scalable, multiplexed pathogen detection. Using CARMEN–Cas13, a multiplexed assay that simultaneously differentiates all 169 human-associated viruses has been developed (Ackerman et al., 2020).
- **DETECTR** (DNA endonuclease-targeted CRISPR trans-reporter): Cas12a acts like Cas13a in this system, and another enzyme, recombinase polymerase amplification, is used as a detection tool to screen for viral infections in cancer (e.g. human papillomavirus 16/18 in lung carcinomas) and to amplify microsamples (Chen et al., 2018b).
- **AIOD-CRISPR** (All-in-One Dual CRISPR–Cas12a) uses Cas12a and two single-guide RNAs to detect HIV and COVID-19 (Ding et al., 2020).

Box 5. Coronavirus disease 2019

Thanks to the flexibility of use of CRISPR, when the COVID-19 pandemic started scientists immediately adapted various CRISPR-based tools to provide alternative diagnostic methods and offer potential therapeutic strategies.

COVID-19 detection

- **SHERLOCK.** This system was adapted by the Broad Institute to detect the virus in about 1 hour. The test can detect the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) S protein gene and *Orf1ab* gene. The tool has not yet been tested in clinical trials (Broad Communications, 2020).
- **CARMEN.** While this system was being developed, a new test for SARS-CoV-2 was rapidly incorporated. Using a single mChip, more than 400 samples can be tested in parallel against the coronavirus panel.
- **DETECTR.** A team from the University of California and Mammoth Biosciences adapted the tool to COVID-19. Since DETECTR is aimed at DNA-based detection, an additional step was added in which virus RNA (N and E genes) is copied into DNA and amplified. The DETECTR method has been validated using COVID-19 patients' samples, but it has not yet been approved by US Food and Drug Administration for clinical use (Broughton et al., 2020).
- **AIOD-CRISPR.** See **Section 4.4.1**.
- The search for clinical trials also identified one trial dedicated to COVID-19 diagnostics based on CRISPR/Cas by the Shenzhen Second People's Hospital, China (Chinese Clinical Trial Registry, 2020). No clear details on the method are provided in English.

COVID-19 therapy

The scientific community is already working on the development of tools for the elimination of COVID-19 using CRISPR.

- **CARVER** (Cas13-Assisted Restriction of Viral Expression and Readout). This system, combined with SHERLOCK, was developed by the Broad Institute to attack RNA-based viruses (Freije et al., 2019).
- **PAC-MAN** (Prophylactic Antiviral CRISPR in huMAN cells). This system, developed by Stanford University, uses Cas13d to attack influenza viruses and SARS-CoV-2-like particles (Abbott et al., 2020).

3.5.2. Data gaps in human health

Data on NGT applications in humans derived from two sources: clinical trials databases and scientific literature. As explained in the **Chapter 2**, data on clinical trials can be considered close to exhaustive, since several databases were consulted, from several countries worldwide that are considered relevant to the sector. In addition, literature was consulted to identify representative examples of all possible different (and promising) uses of NGTs to tackle human diseases (excluding basic research purposes, as explained in the **Chapter 1**). Therefore, the information derived from the literature can be considered representative but not exhaustive. This, of course, must be taken into consideration when analysing the figures in this chapter.

4. Conclusions

NGTs in Group 1, especially those based on CRISPR, are being actively and increasingly used in agri-food, industrial and medicinal sectors, in all the organisms analysed (plants, mushrooms, animals, microorganisms and human cells).

Thanks to its flexibility, affordability and ease of use, CRISPR is opening the doors to several new possibilities in terms of organisms in which it can be used and traits that can be obtained, and it is also reaching many players worldwide.

A big set of data has been collected for this study, and it offers a very good representation of the current and potential use of NGTs for commercial purposes. Currently, few applications are marketed worldwide (one plant product, one microorganism for release into the environment and several microorganisms used for contained production of commercial molecules), but there are about 30 identified applications (in plants, animals and microorganisms) at a pre-commercial stage in the pipeline that could reach the market in the short term (within 5 years). If the applications identified in this study as being at the advanced R & D stage become commercial in a decade, by 2030 over a hundred plants and several dozen animals and medical applications could be on the market. It is safe to say that most of these applications commercialised by 2030 would be based on Group 1 techniques (CRISPR) and some on Group 2 techniques. The maturity of Group 3 and 4 applications in terms of commercial release is lower.

A sector of particular interest is the use of microorganisms for industrial purposes, where NGT applications cannot be singled out (as in plants, animals or clinical trials) because the NGTs are already being taken up and embedded in the routine genetic toolbox for strain improvement. It appears that the use of NGTs is already a reality in many cases, probably facilitated by the contained use of microorganisms and the fact that the final product is not the target. Within the bioeconomy, it is very likely that uptake of NGTs will be faster in relation to microorganisms producing industrial bio-based molecules (e.g. biofuels). The field of pharmaceutical and cosmetic products derived from microorganisms represents a data gap in this study, but we believe that it is also a very important field of application of NGTs in products that may have already reached the market.

In the medicinal sector, there are promising uses of CRISPR-based techniques and other NGTs to tackle several human diseases, and in many cases applications have already reached patients, in phase I and phase I/II clinical trials. In addition, thanks to the flexibility of CRISPR/Cas, both in terms of sequence specificity and different uses, it is already being applied to the search for solutions for rapid detection of COVID-19 and also in some therapeutic options against the disease.

Our database of NGT applications points to the United States and China as the most frequent countries of origin, particularly in the stages closest to market. The EU, particularly Germany and France, is also active in the use of NGTs. Thanks to the flexibility and affordability of NGTs (especially CRISPR), several developing countries are also active in the field, mostly in the agricultural sector.

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Abbreviations

ADAR	Adenosine deaminase acting on RNA
AIOD-CRISPR	All-in-One Dual CRISPR-Cas12a
ARM	Alliance for Regenerative Medicine
CAR T cells	Chimeric antigen receptor T cells
CARMEN	Combinatorial Arrayed Reactions for Multiplexed Evaluation of Nucleic acids
CARVER	Cas13-Assisted Restriction of Viral Expression and Readout
Cas	CRISPR associated protein
CGIAR	Consultative Group for International Agricultural Research
COVID-19	Coronavirus disease 2019
CRISPR	Clustered regularly interspaced short palindromic repeats
CRISPRi	CRISPR interference
CTNBio	National Technical Commission on Biosafety of Brazil
DETECTR	DNA endonuclease-targeted CRISPR trans-reporter
DNA	Deoxyribonucleic acid
DSB	Double strand break
EFFAB	European Forum of Farm Animal Breeders
EFPIA	European Federation of Pharmaceutical Industries and Associations
EPSO	European Plant Science Organisation
EU	European Union
EuropaBio	European Association for Bioindustries
EU-SAGE	Network of European Sustainable Agriculture through Genome Editing
FSANZ	Food Standards Australia New Zealand
GABA	Gamma-aminobutyric acid
GM	Genetically Modified
GMO	Genetically Modified Organisms
HIV	Human immunodeficiency virus
ISF	International Seed Federation
JRC	Joint Research Centre of the European Commission
NARO	National Agriculture and Food Research Organization
NGT	New Genomic Technique
ODM	Oligonucleotide directed mutagenesis
OGTR	Office of the Gene Technology Regulator
PAC-MAN	Prophylactic Antiviral CRISPR in huMAN cells
PRI	Pharmabiotic Research Institute
PRKAG2	5'-AMP-activated protein kinase subunit gamma-2
R & D	Research and Development
RdDM	RNA directed DNA methylation
RESTORE	Recruiting endogenous ADAR to specific transcripts for oligonucleotide-mediated RNA editing
RNA	Ribonucleic acid
SDN	Site-directed nuclease
SHERLOCK	Specific high sensitivity enzymatic reporter UnLOCKing
SNIF	Summary Notification Information Format
ssDNA	Single strand DNA
TALEN	Transcription activator-like effector nuclease
USDA	U.S. Department of Agriculture
USDA-APHIS	Animal and Plant Health Inspection Service. U.S. Department of Agriculture
ZFN	Zinc-finger nucleases

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Annex 1. Example of questionnaire for data collection on new genomic technique applications

New genomic techniques: global market applications

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BACKGROUND INFORMATION

As the internal provider of scientific and technological advice to the European Commission, the Joint Research Centre (JRC) is carrying out a review of current applications of new genomic techniques (NGTs) that are marketed worldwide, or are at a near-market development stage, in agricultural, pharmaceutical and industrial fields.

Examples of techniques include (1) genome editing techniques such as CRISPR, TALEN, zinc-finger nucleases, meganuclease techniques, prime editing, etc., which techniques can lead to mutagenesis and some of them also to cisgenesis, intragenesis or transgenesis; (2) mutagenesis techniques such as oligonucleotide-directed mutagenesis (ODM); (3) epigenetic techniques such as RNA-directed DNA methylation (RdDM). Conversely, techniques already in use prior to 2001, such as *Agrobacterium*-mediated techniques or gene guns, are not considered NGTs.

The JRC is building a database of NGT applications based on several data sources. This questionnaire is directed to organisations that are developing NGT crops that are (1) commercial, (2) close to commercialisation, (3) at the advanced R & D stage or (4) at the early R & D stage, and for which future commercialisation is envisaged.

The JRC is committed to ensuring the protection of any commercial interests of a natural or legal person. Therefore, participants should indicate which information should be treated as confidential. Personal data, if any, will be protected pursuant to Regulation (EU) 2018/1725.

QUESTIONNAIRE

ON THE COMPANY/INSTITUTION

- Name of the company/institution:
  _____
- Country in which the company/institution is based (headquartered)
  _____
- Size ([8]):
  - Microenterprises: fewer than 10 persons employed;
  - Small enterprises: 10 to 49 persons employed;
  - Medium-sized enterprises: 50 to 249 persons employed;
  - Large enterprises: 250 or more persons employed.
  - Other: spin-off, PPP, public institute, etc.

ON THE NGT PRODUCT(S)

- Organism (plant, animal or microorganism) in which the technology is used:
  _____
- Technology (see the definition in the background information)
  _____
- Outcome of the use of the technology:
  - Mutagenesis (including small deletion or insertions)
  - Cisgenesis/intragenesis
  - Transgenesis
  - Epigenetic modification
  - Other. Please specify:_____
- Trait (or trait category e.g. insect resistance, higher yield, etc.)
  _____
- Time to market:
  - **Commercial stage.** NGT applications currently marketed in at least one country worldwide.
  - **Pre-commercial stage.** NGT applications ready to be commercialised in at least one country worldwide but not yet on the market (commercialisation mainly depends on the developer's decision and a 5-year horizon is estimated).
  - **Advanced R & D stage.** NGT applications at a late stage of development (field trials in the case of plants, in/ex vivo clinical trials in the case of medical applications) and likely to reach the market in the medium term (i.e. by 2030).
  - **Early R & D stage.** NGT applications at proof of concept stage (i.e. testing gene targets for trait enhancement of commercial interest).
- Related publication(s), if any:
  _____

([8]) Company sizes as defined by Eurostat (https://ec.europa.eu/eurostat/statistics-explained/index.php/Glossary:Enterprise_size).

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