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Review Article

Next biotechnological plants for addressing global challenges: The contribution of transgenesis and new breeding techniques

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ABSTRACT

The aim of this survey is to identify and characterize new products in plant biotechnology since 2015, especially in relation to the advent of New Breeding Techniques (NBTs) such as gene editing based on the CRISPR-Cas system. Transgenic (gene transfer or gene silencing) and gene edited traits which are approved or marketed in at least one country, or which have a non-regulated status in the USA, are collected, as well as related patents worldwide. In addition, to shed light on potential innovation for Africa, field trials on the continent are examined. The compiled data are classified in application categories, including agronomic improvements, industrial use and medical use, namely production of recombinant therapeutic molecules or vaccines (including against Covid-19). The data indicate that gene editing appears to be an effective complement to 'classical' transgenesis, the use of which is not declining, rather than a replacement, a trend also observed in the patenting landscape. Nevertheless, increased use of gene editing is apparent. Compared to transgenesis, gene editing has increased the proportion of some crop species and decreased others amongst approved, non-regulated or marketed products. A similar differential trend is observed for breeding traits. Gene editing has also favored the emergence of new private companies. China, and prevalently its public sector, overwhelmingly dominates the patenting landscape, but not the approved/marketed one, which is dominated by the USA. The data point in the direction that regulatory environments will favor or discourage innovation.

Introduction

The development of gene transfer technologies (transgenesis) has hugely facilitated basic and applied research since the 1980s and some of its products have been marketed from the mid-1990s [1]. The term 'classical' is used here for these techniques. Gene editing applied to plants could be the next revolution and includes sequence-specific nucleases, such as Zinc Finger Nuclease, TALENs (Transcription Activator-Like Effector Nucleases), CRISPR-Cas systems (Clustered Regularly Interspaced Short Palindromic Repeats) and also Oligonucleotide-Directed Mutagenesis (ODM) technologies [2]. Overviews of the use of CRISPR-based gene editing in plants, its challenges and prospects, including regulatory constraints have been published recently [3–9]. While discussing the legal situation and implication of gene editing in the EU, a report by German scientific authorities [10] and an article by Purnhagen and Wesseler [11] also listed a number of applications and potential applications of these technologies.

In the present review, which takes a different angle, recently approved or marketed innovations in plant biotechnology have been compiled and classical transgenesis is distinguished from gene editing. Regarding classical transgenesis, gene transfer is also distinguished from gene silencing *via* anti-sense or RNA interference methods, collectively termed RNAi [12]. As a complement to identify the most recent innovations, patents using classical transgenesis or the CRISPR-Cas system in plants have been compiled. Examination of field trials could also shed light on original research projects and the focus here is on Africa since it

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Abbreviations: BBTV, banana bunchy top virus; CBDS, Cassava Brown Streak Disease; CBI, Corporate Business Information; CRISPR-Cas, Clustered Regularly Interspaced Short Palindromic Repeats; ISAAA, International Service for the Acquisition of Agri-biotech Applications; ODM, Oligonucleotide-Directed Mutagenesis; TALEN, Transcription Activator-Like Effector Nuclease; USDA-APHIS, US Department of Agriculture – Animal and Plant Health Inspection Service.

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has “the biggest potential to reap benefits associated with modern agronomic biotechnology” according to the International Service for the Acquisition of Agri-biotech Applications (ISAAA) [13]. The goals are to document how biotechnology could provide tools to address global challenges in agriculture and, in regard to gene editing, to examine whether it involves new plants, new traits or new actors, and whether classical transgenesis techniques and gene editing are complementary or competitors.

Material and methods

Approved, non-regulated and marketed biotechnological plants

Biotechnological plant varieties approved for commercial use by regulation agencies in the world or already marketed in at least one country were compiled from the ISAAA GM database [14]. This includes varieties which obtained ‘deregulated’ status in the USA after risk evaluation [15]. In addition, in the USA, products exempt from the regulations (non-regulated) were obtained from the ‘Am I regulated’ US Department of Agriculture - Animal and Plant Health Inspection Service (USDA-APHIS) database [16]. Websites of potential developers are another source of information utilized. When ‘Corporate Business Information’ (CBI) was not available, the data were annotated as "other gene editing (not disclosed)". In addition, plant lines expressing non-pesticidal ‘new proteins’, evaluated by the US Food and Drug Administration (FDA) for food safety, were compiled from [17]. Plants at the Research and Development (R&D) stage are not included in the compilation of the *Approved, non-regulated or marketed new biotechnological plants* section of Results.

Plant biotechnology patents

Patents related to inventions based on classical transgenesis (gene transfer or RNAi) or the CRISPR-Cas system were obtained using the Orbit Intelligence database [18]. The search query equation is shown in the patent Supplementary file. This search was limited to the 45 major species collected in the ‘approved and marketed’ section. Patent records were regrouped into patent families (containing all extensions of a given invention) including patent titles, abstracts, inventors, applicants, priority dates and the various reference numbers.

Manual sorting

Compiled ‘approved/non-regulated/marketed’ products and patents were sorted into technical categories (classical transgenesis, subdivided into gene transfer or RNAi, and gene editing, limited to CRISPR for patents), or into thematic categories (Agronomy/Nutrition, Industrial, Biopharmaceuticals, plus Technical Improvement for patents) and further subdivided.

Field trials in Africa

Relevant information was found on the websites of the research projects/programs, on those of the Ministry of Agriculture or the agency responsible for biotechnology regulation.

Results

Approved, non-regulated or marketed new biotechnological plants

The compilation, encompassing January 2015 to October 2020, is presented in two datasheets of Supplementary file 1, with a total of 219 entries (comprising 152 related to agriculture, 20 to industrial use of which 2 are stacked (i.e. genetic traits grouped in a single variety; also called pyramided) with an improved agronomic trait, 39 to therapeutic use and 10 non-available data (annotated as ‘nd’, not disclosed). Some entries (most commonly herbicide tolerance with another trait) are composed of stacked individual events. Furthermore, some individual events harbour more than one trait. In order to perform a more refined and quantitative analysis (see following sections), all stacked events and the traits combined in certain events were individualized. For a meaningful overview of the proportion of each technique (Fig. 1), redundant traits were counted only once, leading to a total of 222 individualized non-redundant traits. This individualized dataset is analyzed in the following sections. Agronomic traits were classified into subcategories: herbicide tolerance, biotic stress, abiotic stress, or other agronomic, harvest and post-harvest traits, and also nutritional traits (see column C of Supplementary file 1).

New biotechnological plants with improved agronomic or nutritional features

This category comprises 165 individualized traits, representing 74 % of the total.

Herbicide-tolerant (HT) biotechnological plants

HT traits, representing 24 % (40 traits) of improved agronomic/nutritional features, are mostly designed for two herbicides: glyphosate (Roundup-Ready technology of Monsanto, Saint Louis, MO, USA) and glufosinate (LibertyLink technology of Bayer, Leverkusen, Germany). To date, transgenic carnation tolerant to sulfonylurea herbicide, transgenic cotton tolerant to Oxynil, transgenic maize or soybean tolerant to 2–4 D and Dicamba have been marketed. These traits are mostly transgenic. A rice and a flax HT variety (CBI not disclosed) obtained using ODM technology by Cibus (San Diego, CA, USA) has obtained a non-regulated status in the USA. These traits are compiled in Table 1 of Supplementary file 2 along with biotic stress resistance traits, since both trait categories are increasingly stacked.

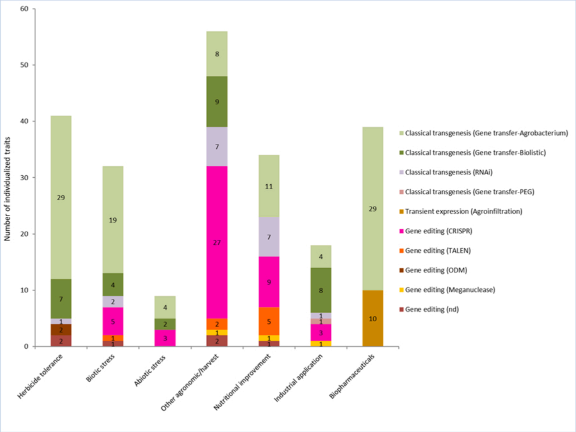

Fig. 1. Relative importance of various techniques used to produce recently approved, non-regulated or marketed engineered plant varieties per application categories and subcategories.
Classical transgenesis (as defined in the text) is sub-divided into gene transfer (*i. e.* of a coding sequence) and gene silencing (RNAi). Gene transfer products are further sub-divided depending on the technique used (either *Agrobacterium,* biolistics or PEG mediated-protoplast transformation). The various gene editing techniques are differentiated as indicated. ‘gene editing (nd: ‘not disclosed’)’ means that details on the techniques are not publicly available. Values correspond to the number of individualized traits, as explained in the text and detailed in the various tables of Supplementary file 2. ‘recently’ means during the 2015–2020 period. ‘non-regulated’ applies in the USA.

Biotechnological plants with biotic stress resistance traits

Biotic stresses, representing 19 % (31 traits) of improved agronomic/nutritional features, are caused by pests such as insects, nematodes, microscopic fungi, bacteria and viruses (see Table 1 of Supplementary file 2). Fig. 2a shows the number of these traits compared to the more numerous HT traits.

All types of techniques are represented, except meganucleases, namely gene transfer by classical transgenesis (19 traits transferred *via Agrobacterium tumefaciens* and 4 by biolistics), gene silencing by RNAi (2), or gene editing (7), 5 of which using CRISPR-Cas9, one TALEN and one whose technical details are not disclosed. A total of 12 plant species are present (Fig. 2b). Currently such transgenic traits in cotton, cowpea, maize and soybean are on the market and sugarcane traits are approved for cultivation (in at least one country). Transgenic potato, rice, sugarcane and tomato lines have been approved for cultivation in at least one country. Over the period 2015–2020, gene editing did not lead to products that were brought onto the market in this pest resistance subgroup (some have a non-regulated status that renders them marketable), whereas transgenic varieties are commercialized.

Regarding resistance to fungal, viral or bacterial diseases, one can cite Innate™ potatoes resistant to the fungus *Phytophthora infestans* (transgenic for this trait) which are marketed by J.R Simplot Company (Boise, ID, USA). Varieties of canola obtained by Cibus *via* gene editing for fungal disease resistance, citrus by Soilcea (Tampa, FL, USA) *via* CRISPR-Cas for viral disease resistance, maize by DuPont Pioneer (Johnston, IA, USA) *via* CRISPR-Cas for fungal resistance, and wheat by Calyxt (Roseville, MN, USA) *via* CRISPR-Cas for fungal resistance are approved for cultivation in at least one country, as is a tomato line obtained *via* RNAi by Nexgen Plants Pty Ltd (Brisbane, Australia) for viral disease resistance. Soybean lines with improved resistance to Soybean Cyst Nematode obtained by Evogene (Rehovot, Israel) using CRISPR-Cas are deregulated in the USA.

Biotechnological plants designed to overcome biotic stresses were obtained primarily by major international seed companies as shown in Fig. 2c. Regarding ‘new players’, out of 6 companies 4 are based in the USA. The universities / public research centers in Fig. 2c are located in USA (Iowa State University, Ames; University of Georgia, Griffin; University of Missouri, Columbia) and Brazil (Centro de Tecnologia Canavieira, Piracicaba, State of São Paulo). Also worth mentioning is the African Agricultural Technology Foundation, a brokering organization that works with different public and private sector technology developers from around the world and facilitates cooperation and implementation in the African context.

Biotechnological plants resistant to abiotic stresses

This subcategory represents 5.5 % (9 traits) of the total of improved agronomic/nutritional features. Relevant abiotic stresses are drought and/or salinity tolerance developed in 4 species. The techniques used are transgenesis (4 by gene transfer *via Agrobacterium* and 2 using biolistics) and CRISPR-Cas9 (3). The data are summarized in Table 2 of Supplementary file 2. Of these traits, one maize line was obtained by CORTEVA (Wilmington, DE, USA) and one by Monsanto, one soybean

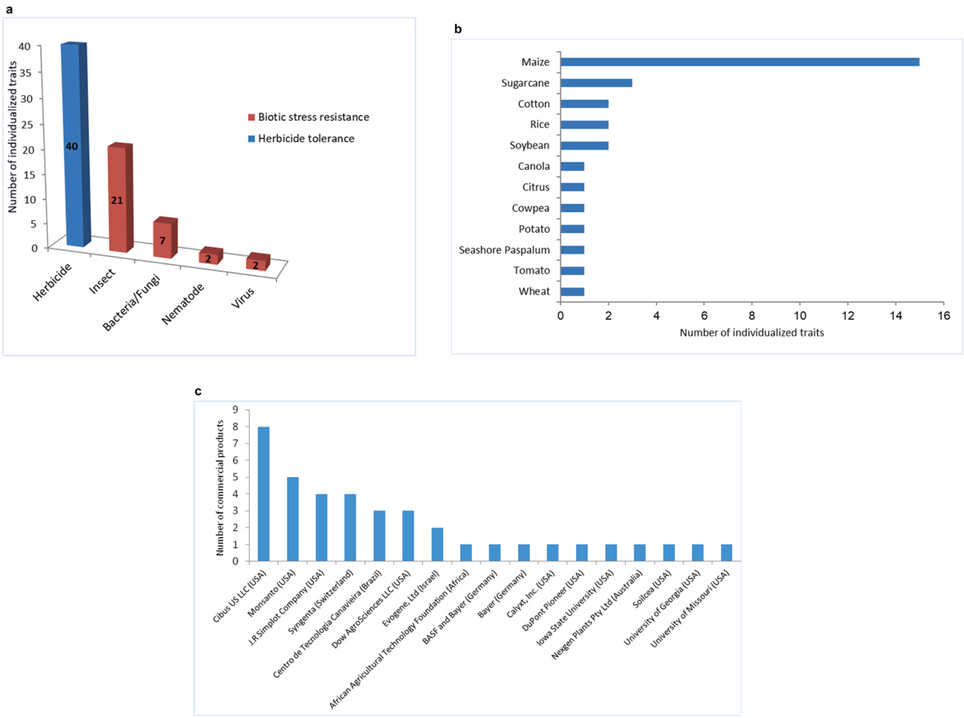

Fig. 2. New biotechnological plants modified to resist biotic stresses.
a. Distribution of traits per application subgroup.
Various biotic stresses (resistance to some insects, nematodes, bacterial or fungal diseases, or viral diseases) are distinguished, and compared to the number of herbicide tolerance traits.
b. Distribution of biotic stress resistance traits per species.
c. Distribution per biotech trait owner.
By choice, considering recent fusions or acquisitions, the current name of the company is used. The term ‘Biotech companies’ gathers all developers producing at least one event/trait. Abbreviation: Seita for Seita Imperial Tobacco.
Values correspond to the number of individualized traits as detailed in Table 1 of Supplementary file 2, except for panel c where values are based on the compilation of commercial products (approved for cultivation, non-regulated or on the market) as detailed in Supplementary file 1.

line by Indear (Rosario, Argentina), one by USDA ARS (St Paul, MN, USA) and one by Verdeca, a joint venture of Arcadia Biosciences (Davies, CA, USA) and Bioceres Crop Solutions Corp. (Santa Fe, Argentina), one rice line by Texas A&M University (College Station, TX, USA), and one miscanthus line by Ceres (Thousand Oaks, CA, USA).

Biotechnological plants with other agronomic, harvest and post-harvest traits

This subcategory includes various improved features of direct interest for farmers, either for crop production or harvest/post-harvest quality (Table 3 of Supplementary file 2**)**. It represents 32 % (53) of the total of improved agronomic/nutritional features. One type of trait is relevant to biomass production prior to harvest (increased yield) or pollination control (male sterility, fertility restoration). A second is relevant to harvest stage (delayed ripening/senescence, flower or fruit color) and a third to post-harvest features (shelf-life, non-browning, reduced black spot formation after bruising, delayed fruit softening). These traits were obtained either by classical gene transfer (*via Agrobacterium:* 8 traits; biolistics: 9 traits), RNAi gene silencing (7) or gene editing (CRISPR-Cas9: 27 traits; TALEN: 2; meganuclease: 1; undisclosed details: 2). The 23 relevant species are shown in Table 3 of Supplementary file 2. Developers are primarily, in descending order, from USA, Germany, Japan, USA + Canada and Israel. One can note that the transgenic variety of chrysanthemum was obtained by Suntory Flowers Limited (Tokyo, Japan) and is non-regulated for importation in the USA.

Biotechnological plants with nutritional improvements

This subcategory represents 19 % (32) of the total of improved agronomic/nutritional features. As shown in Table 4 of Supplementary file 2, nutritional improvements for human or animals are either modifications in oil/fatty acid, carbohydrate, lignin, protein or vitamin A content, phytase production, or reduced content of asparagine and reducing sugar to lower production of acrylamide upon frying (see also [19]). These traits were obtained by either classical transgenesis, namely gene transfer (*via Agrobacterium*, 11 traits) or RNAi gene silencing (7), or by gene editing such as TALEN (5), CRISPR-Cas (9), meganuclease (1) and undisclosed gene editing (1). They relate to 12 species: soybean (8 traits), potato (7), canola (5), maize (3), alfafa (2), bahiagrass (1), *Brassica juncea* (1), cotton (1), pea (1), pineapple (1), sugarcane (1), and wheat (1).

RNAi was used to produce potato lines with, amongst other traits (see above: reduced black spot formation), a reduced potential for acrylamide formation upon cooking. The latter trait will also reduce browning and formation of bitter flavors after frying. Various potato varieties marketed under the generic name Innate™ (see above) contain the same gene construct introduced as distinct events in a given Elite genetic background or the same gene construct introduced in different backgrounds.

TALEN technology was used to develop one alfalfa line (low lignin content), 2 soybean lines (modified oil/fatty acid content) and one wheat line (high fiber content). Meganuclease was used in maize for animal nutrition and processing industries. CRISPR-Cas9 was used to create a pennycress line (modified oil/fatty acid content), a soybean line (modified seed composition), a wheat and a tobacco line with unspecified application, a canola line (altered oil content), a pea line (improved flavor), 3 potato lines (2 with reduced glycoalkaloids, 1 with reduced vacuolar invertase which affects reducing sugar content), and a soybean line (high oleic acid content).

Biotechnological plants with industrial applications

Of the total of 18 traits collected (8% of the total individualized traits), 14 were obtained using classical transgenesis for gene transfer (4 *via Agrobacterium*, 8 by biolistics, 1 by PEG-mediated protoplast transformation) or for RNAi (1) and 4 using gene editing (3 by CRISPR-Cas9 and 1 by meganuclease). These plants were created for modified oil/fatty acid for biodiesel or lubrication, modified carbohydrate for bioethanol, improved wood quality and biomass for pulp, paper and wood industries, modified alpha-amylase or starch content, reduced nicotine content and production of recombinant protein for cell culture or research. Eleven species are in this category: loblolly pine (3 traits), poplar (3), maize (2), switchgrass (2), tobacco (2), bahiagrass (1), barley (1), eucalyptus (1), moss (1), pennycress (1) and safflower (1).

Two lines producing recombinant proteins in transgenic barley are commercialized for cell culture and the food industry, respectively, namely the isokine product composed of growth factors and cytokines, and thaumatin, a sweetening protein. Both are created by ORF Genetics (Kópavogur, Iceland). Transgenic eucalyptus and poplar are commercialized for wood improvement by Futuragene (Itapetininga, State of São Paulo, Brazil) and ArborGen (Ridgeville, SC, USA). The transgenic safflower for oil improvement, for products ranging from lubricant for motor vehicles to cosmetics [20], is marketed by Go Resource Pty Ltd (Melbourne, Australia). Five transgenic maize lines are commercialized: one with modified alpha-amylase for bioethanol production (Enogen trait), 2 with recombinant protein production for cell culture and research (avidin and TrypZean™) and 2 with recombinant enzyme production (cellobiohydrolase and endocellulase) for the paper industry.

Three approved lines in this category are not currently marketed. Arcadia Bioscience (Davies, CA, USA) have created a transgenic barley producing a recombinant alanine aminotransferase for cell culture and research, approved by the USA FDA in 2015. Vector Tobacco Inc (Morrisville, NC, USA) obtained a transgenic tobacco with low nicotine content, approved in 2002, but not marketed. 22nd Century Group (Williamsville, NY, USA) created a reduced nicotine content cigarette (Moonlight™) which received authorization for commercialization from the FDA in 2019. The company has also created VLN™ cigarettes, containing 95 % less nicotine, which are currently under review by the FDA.

Biotechnological crops to produce biopharmaceuticals

Traits are included here for which products are involved in at least pre-clinical or clinical trials. This is admittedly an arbitrary choice, but since these plants have a first level of authorization (cultivation), they show the closest correspondence with the selection criteria for the previous categories (at least an approval for cultivation). Potential biopharmaceutical traits were primarily screened from developers' websites. Consequently, a number of the identified products are only at an R&D stage and have not been included in the analyses but added as an Appendix in the vaccine/therapeutics datasheet of Supplementary file 1.

Following these selection criteria, this category represents 39 traits (17.6 %) of the total individualized traits. The prevalent technologies are stable transgenesis and transient expression following agro-infiltration of gene constructs. Fig. 3 presents the species, Table 6 of Supplementary file 2 shows the developers, and Table 7 of Supplementary file 2 lists the diseases and the corresponding vaccines or therapeutic molecules.

Biotechnological crops to produce vaccines

Transgenic plants have long attracted interest for production of injectable or edible vaccines [21,22]. More than 97 experimental vaccines have been produced by molecular farming [23] and 28 in the present dataset (2015–2020, updated March 2021). These 28 products correspond to a total of 15 different vaccine types.

The biotech company Medicago (Quebec, Canada) reported promising results for an influenza vaccine [24]. This partly successful vaccine was developed in *Nicotiana benthamiana*, the species of choice to transiently express genes by leaf agroinfiltration. However to date, no plant-based vaccine is on the market. During the 2015–2020 period considered here, two clinical trials in phase I are noteworthy (one rotavirus vaccine produced in *N. benthamiana* by Medicago, and one malaria vaccine produced in *N. tabacum* by Fraunhofer Center for

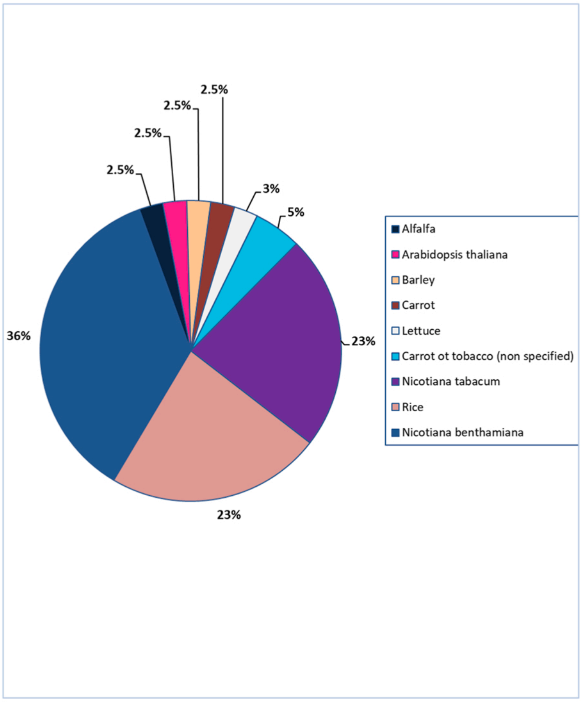

Fig. 3. Distribution of plant species for biotechnological medical applications, approved for (pre)clinical trials or marketing during the 2015-2020 period.
Techniques used to develop vaccines and therapeutic molecules against various diseases including Covid-19 are agroinfiltration for transient expression and stable transgenesis.
Values correspond to the number of individualized traits as explained in the text and as detailed in Table 7 of Supplementary file 2.

Molecular Biotechnology (Newark, DE, USA). Results of a phase III trial were obtained for the Medicago influenza vaccine in 2020 (registration in progress in 2021).

Regarding the Covid-19 pandemic, 3 SARS-CoV-2 vaccines have been developed by Medicago (phase II-III) in association with GSK (Stevenage, UK), iBio (Newark, DE, USA; preclinical trials) and Beijing CC-Pharming (Beijing, China) in *N. benthamiana,* and Kentucky BioProcessing (Owensboro, KY, USA; phase I) with British American Tobacco (London, UK) in *N. tabacum*. All use transgenesis (*Agrobacterium*) or biolistics to introduce the gene of interest. Medicago is using SARS-CoV-2 Virus-Like Particles to develop a vaccine [25].

Biotechnological crops to produce therapeutics

Therapeutic proteins (Supplementary file 2 - Table 7) include treatments for inflammatoryand diarrheal disease or cancer, but few products have been commercialized to date. Elelyso, a human taliglucerase alfa produced in carrot cells, has been developed by Protalix Biotherapeutics (Karmiel, Israel) and commercialized by Pfizer (New York, USA) and is prescribed for Gaucher type I disease. ORF Genetics commercializes Bioeffect, an ‘anti-aging’ skincare with recombinant epidermal growth factor (EGF) produced in barley. CollPlant (Rehovot, Israel) commercializes a recombinant human type I collagen, Arthrex ACP™ Tendo, produced in tobacco. Merck KgaA (Darmstadt, Germany) commercializes a recombinant bovine aprotinin produced in tobacco, A6103, to reduce bleeding during complex surgery. One can also mention two transgenic carrot or tobacco lines producing a recombinant human tumor necrosis factor receptor II (TNFR2) for inflammatory bowel disease, OPR-106, and a recombinant human deoxyribonuclease I (DNase I) for cystic fibrosis treatment, ‘PRX-110′. These molecules, developed by Protalix Biotherapeutics are currently at the clinical trial stage (phase II).

Transgenic rice producing recombinant lactoferrin, developed by Ventria Biosciences (Fort Collins, CO, USA) for therapy of inflammatory bowel disease is stated to be in clinical tests (phase II) [26]. Two other transgenic rice lines were tested in humans between 2015 and 2020: one to reduce pollen allergy was developed by Japan public laboratories (Jikei University School of Medicine, Tokyo; National Institute of Agrobiological Sciences) and another which produces recombinant human serum albumin, OsrHSA, was developed by Oryzogen (Hubei, China) and was approved for clinical trials in 2017 in China and in 2019 in the USA [27]. In addition, transgenic *N. tabacum* (monoclonal antibodies CO17−1A × BR55 for colorectal cancer [28]), *N. benthamiana* (various candidates including fusion of the endostatin derived E4 antifibrotic peptide to the hinge and heavy chain of human IgG1 for fibrotic disease [29]), and rice (proinsulin-transferrin fusion protein for diabetes [30], resveratrol-enriched for metabolic syndrome [31]) were tested on animals between 2015 and 2020 (preclinical test).

Recent patent landscape of biotechnological plants

Innovations are usually patented at the earliest possible stage to ensure intellectual property. Thus, a patent landscape will provide information far upstream of potential marketing. The present study gathers together 1736 patents regarding plants engineered by gene transfer, RNAi, or gene editing *via* a CRISPR-Cas system (Supplementary file 3). This compilation covers the period from 2015 to 31st December 2019 as priority date. Earlier patents involving the CRISPR system are available in [32].

Geographical distribution of plant biotechnology patents

China ranks first with 1564 patent families, or 90 % (Table 1 of Supplementary file 4). The USA, although leader in terms of plant biotechnology sales, is far behind with only 3.7 % (65 patent families). Korea which does not allow the growth of transgenics, but imports them, filed 3.2 % (56). EU Member States as a whole represent only 1.2 % (21). These data correspond to the patents publicly available on 16th June 2020, the last database screening. To evaluate the respective patenting weight of each country more accurately, it was necessary to take into account that China publishes many patents before the delay of 18 months after the first priority date (see [32]). Thus, 16th December 2018 corresponds to this 18 month delay before the last database update. Indeed, 407 patents, 402 of which filed by China, were published after 16th December 2018. However, excluding these does not change significantly the balance between China (87.4 % of filed patents) and other countries (USA: 4.7 %, Korea: 4.1 % and Europe: 1.6 %).

Patent landscape according to biotechnology method

Gene transfer by classical transgenesis remains the most widely used method worldwide (75 % of the compiled patents). CRISPR-Cas9 was used in 14 % of these patents while RNAi is represented in 11 %. Patents concerning the CRISPR technique have been filed mostly by China (92.5 %, 235 patent families), followed by USA (4%, 10 families), Europe (2.4 %, 6 families), Saudi Arabia (0.8 %, 2 families) and Korea (0.4 %, 1 family). China’s place is preponderant for all techniques. The evolution of patents per year according to technique is discussed below.

Distribution of biotechnology patents by category

To refine this analysis, patents were sorted manually into four categories, three of which correspond to those examined above, namely agronomy/nutrition (78 %), industrial applications (paper industry, wood production processing, biofuel, oil production, resistance to pollutants, dietary supplement production, or cigarette quality; 4%), and biopharmaceuticals (production of recombinant proteins or of vaccines; 2%). The fourth category was termed ‘technical improvement’ (of transgenesis or gene editing, new promoters or screening methods; 16 %).

Classical transgenesis is the majority in each case, particularly for the biopharmaceutical and industrial categories (100 % and 91 %, respectively). The CRISPR technique takes a significant but not overwhelming share of the technical improvement category (18 %; Fig. 4a). This indicates that classical transgenesis is still considered to be a useful technique and justifies further investments.

Regarding the most numerous category (agronomy/nutrition), resistance to biotic or abiotic stress represents a total of 57 % of these patents (Fig. 4b). Patents related to abiotic stress mostly concern drought (38 % of this subcategory) and salinity tolerance (32 %), whilst 8% concern resistance to low temperature (Fig. 4c). Others are related to heavy metal, nutritional, heat, flooding and osmotic stresses. Herbicide tolerance represents only 5%, a possible explanation being that many such patents were already filed before and the corresponding varieties already approved or marketed. Other patents in this category are related to ‘other agronomic, harvest and post-harvest’ traits (Fig. 4b).

Distribution of biotechnology patents by plant species

Table 2 of Supplementary file 4 shows the number of patent families per plant species and distinguishes among the latter those directly related to the patent claims, those from which the biological material was originally obtained, and those simply used as a model plant. It should be noted that 83 of the compiled patents apply to various species and were not used in the breakdown per species. This table also shows the increase in CRISPR patents when comparing a previous compilation to the present one [32]. Rice is the dominant crop in 36 % of these patents, followed by maize (11 %), tobacco (11 %, half concerning tobacco as a model plant), soybean (8.6 %), cotton (8%), wheat (6%), tomato (5.1 %) and Brassica (all species, 4.6 %). China is the leader for all these major crops (Fig. 5).

Discussion

The proportion of various technologies

To identify recent trends in plant biotechnology, biotechnological events approved or marketed in at least one country have been compiled and 222 individualized non-redundant traits sorted in 45 varieties, of which 70 % were obtained using classical transgenesis for gene transfer or silencing (RNAi, in 8%). Strikingly, although gene editing is often considered as a new biotechnological revolution, the present compilation identifies only 29.7 % of individualized traits obtained by gene editing techniques: 21.6 % by CRISPR-Cas, 3.1 % by TALEN, 1.4 % by

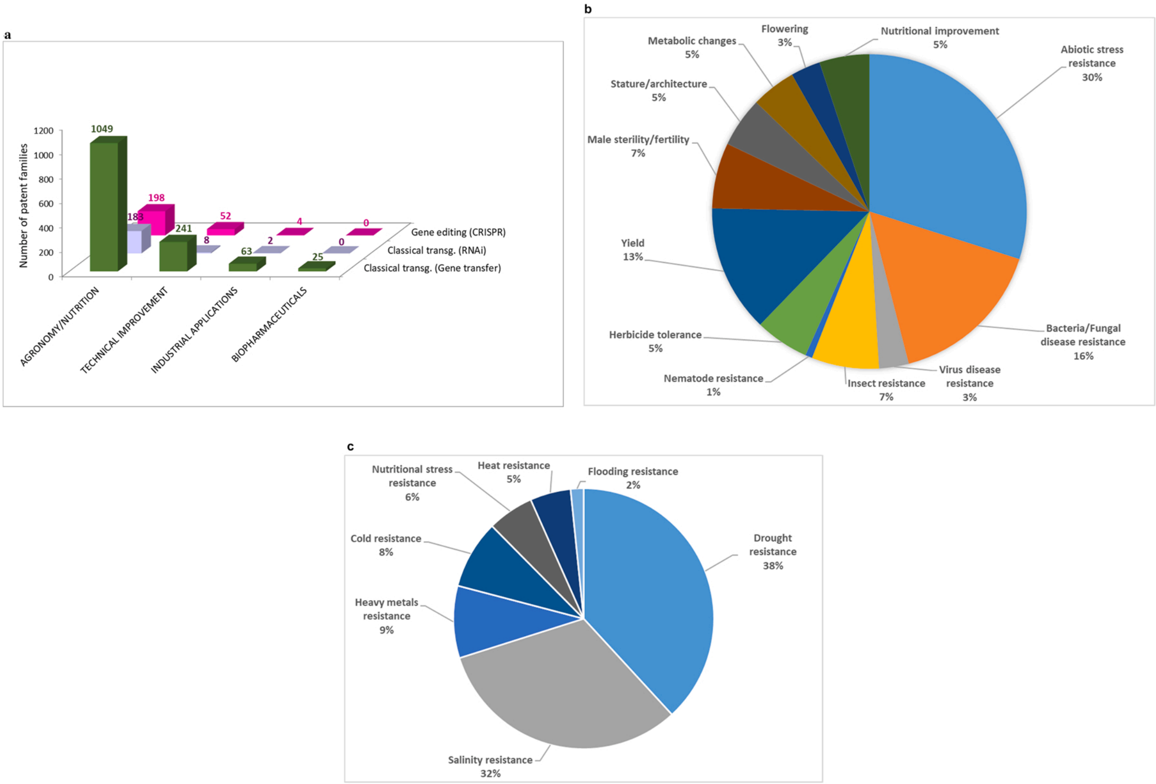

Fig. 4. Distribution of patents related to plant biotechnology and sub-divided in application categories and sub-categories.
Data relate to patents filed between 2015 and 2019 (priority date) and grouped in patent families as presented in Supplementary file 3.
a. Number of biotechnological patents per usage category and techniques.
Classical transgenesis techniques (as defined in the text) are sub-divided into gene transfer (*i.e.* of a coding sequence) and gene silencing (RNAi) and compared to gene editing techniques. The usage categories are ‘agronomy/nutrition’ (which includes herbicide tolerance, resistance to biotic or abiotic stresses, other agronomic, harvest and post-harvest traits and nutritional improvement), ‘industrial’ or ‘biopharmaceutical’ applications, as well as general ‘technical improvement’.
b. Distribution (%) of traits related to agronomy/nutrition.
The biotic stress traits (regrouped in the lower-right part of this pie chart) represent 27 % of the total. The ‘other agronomic, harvest and post-harvest’ traits (regrouped on the left part of this pie chart) represent 31 % of the total.
c. Distribution (%) of traits related to abiotic stress.

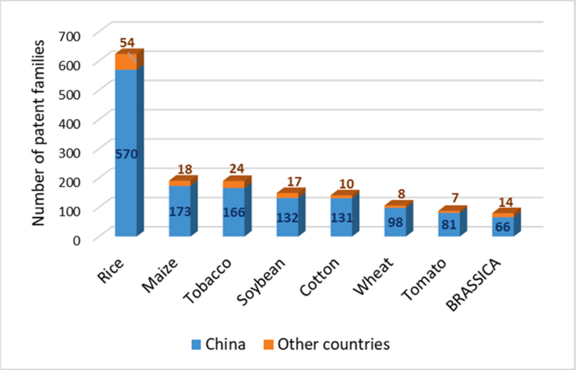

Fig. 5. Comparative number of patents filed by China and other countries per crop species.
Data relate to patents filed between 2015 and 2019 (priority date) and grouped by patent family.

Meganuclease, 0.9 % by ODM and 2.7 % by non-disclosed gene editing techniques (Fig. 1). The distribution of techniques per species is shown in Fig. 6.

Regarding RNAi, potato, sugarcane and tomato lines have been approved for cultivation in the USA. Commercialized RNAi crops (in Canada, USA, Australia and Japan) are:

- non-browning Arctic™ apple by Okanagan Specialty Fruits Inc. (Summerland, BC, Canada),
- Honey Sweet™ plum resistant to Plum pox potyvirus (Sharka) obtained by a public research consortium was marketed in the USA before 2015,
- various Innate™ potato varieties (see above),
- a safflower with enhanced oil content is commercialized by Go Resources Pty Ltd (Melbourne, Australia),
- Oncidium-orchid with modified flower color (by silencing of the phytoene synthase gene), obtained by University of Tsukuba (Tsukuba, Japan), is approved for importation to Japan from USA.

Regarding gene edited crops currently on the market, the sulfonylurea HT canola by Cibus (Falco™) was obtained during a selection program using the company’s proprietary ODM technology RTDS™ (Rapid Trait Development System™) but actually represents a somaclonal variation (see [33] and was not included here. Cibus is also developing gene-edited flax, potato, rice and soybean [34]. A few companies are using site-directed mutagenesis with nucleases. Only one such crop is currently on the market (the Calyno oil soybean produced by Calyxt) with nutritional modifications using TALEN [35]. These companies are promoting their products as “non-GMO”.

The compilation of recent plant biotechnological patents is intended to reveal more recent trends. However, the share of gene editing is also limited (14 %). It should be noted that limiting the survey to the CRISPR-type of gene editing is unlikely to change the conclusion, since this type of gene editing is now overwhelmingly the most popular. Taking into account the fact that 30 % of these CRISPR patents (for the 6 major crops) describe knockout (KO) mutations, gene editing would be expected to supplant RNAi techniques. However, this is not apparent in the data displayed in Fig. 7a, showing the evolution of patents per year: patents based on an RNAi strategy, after a 3-year decline, rebounded in 2019. Furthermore, although gene editing by the CRISPR-Cas technique shows a significant steady increase, gene transfer *via* classical transgenesis is stable year on year (excluding minor fluctuations).

Similarly, as shown in Fig. 7b, in the list of approved/non-regulated/marketed products, the share of classical gene transfer and RNAi is rather stable, ignoring year to year fluctuation. However, the share of gene editing dramatically increased in 2020, primarily due to non-

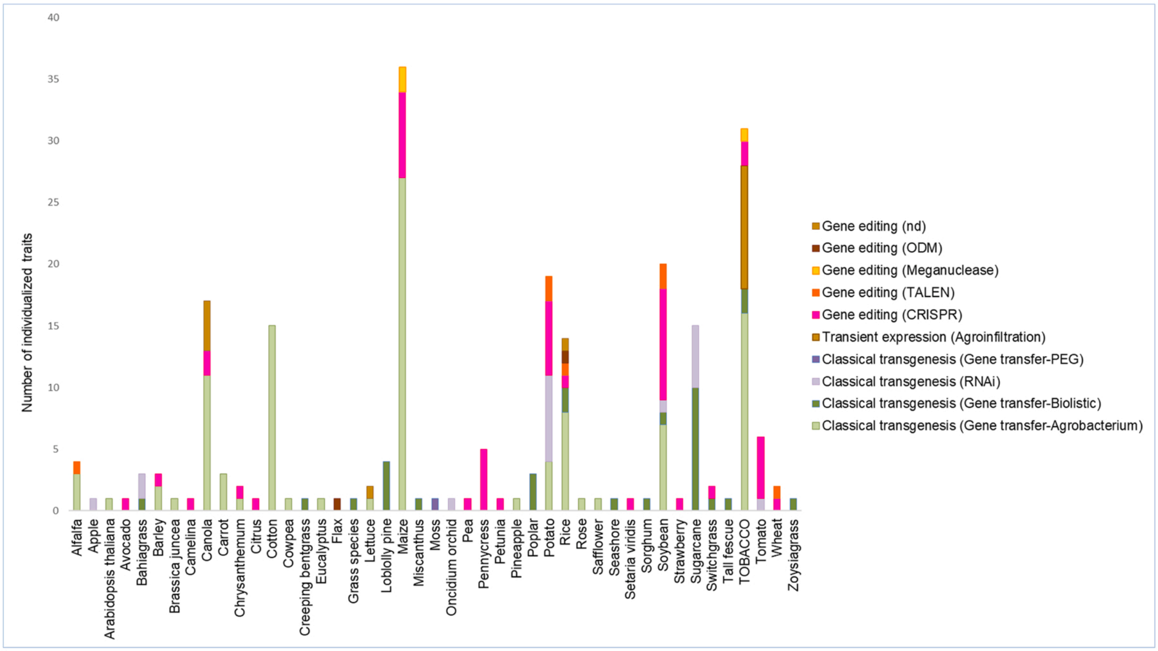

Fig. 6. Distribution of techniques per species for newly approved, non-regulated or marketed biotechnological plants.
Data are from the 2015–2020 period as explained in the text. Techniques are defined as explained in legend to Fig. 1. Values refer to the number of times a given technique was used, and are based on data detailed in the various tables of Supplementary file 2. For biopharmaceutical plants, ‘approved’ means at least for (pre) clinical trials.

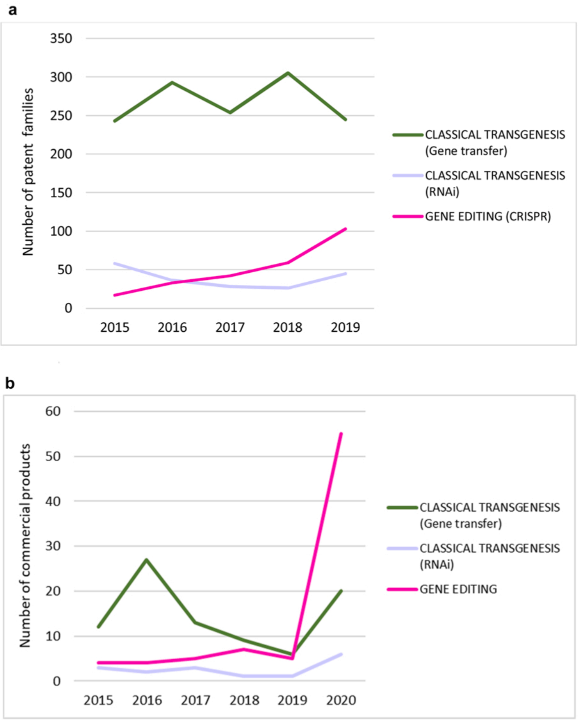

Fig. 7. Evolution of techniques per year in relation to plant biotechnological patent families (a) and approved, non-regulated or marketed products (b). Three major technical choices are compared, namely the use of classical transgenesis for either gene transfer or RNAi, and gene editing (only the most used technique, CRISPR-Cas9, is represented). Patent numbers relate to patents filed between 2015 and 2019 (priority date) and grouped in patent families as presented in Supplementary file 3. Approved/non -regulated (in the USA)/ marketed product numbers relate to data compiled in Supplementary file 1.

regulated products in the USA, which illustrates how favorable regulation can have a positive effect on technology development (for a recent review on regulation, see [36]). This observation can be compared to that in [37] concerning the Argentinian regulatory approach. Since the presented patent data do not include 2020 and are incomplete for 2019 for reasons above, such an increase for gene editing in 2020 cannot be excluded.

Thus, taking into account both patenting and approval for cultivation, it can be concluded that although gene editing techniques are increasingly used, they have not supplanted classical transgenesis. In addition, despite regulatory restrictions in some countries, many transgenic products have reached the market. It remains to be seen which of the 21 gene edited crops with a non-regulated status in the USA will actually enter the market. This will depend on company strategy or market acceptance (see the case of transgenic glyphosate-resistant wheat below). Apparently, none of the gene edited crops with a non-regulated status in the USA actually obtained cultivation authorization outside the USA.

The cases of major crops

An important question is whether new developments (*via* classical transgenesis or recent gene editing techniques) will allow diversification of biotechnological crops, which often implies overcoming regulatory and economic constraints. The case of wheat, the second most-produced cereal after maize and before rice, is interesting since there is currently no biotechnological wheat on the market [38]. Despite its relative recalcitrance to *in vitro* culture and regeneration, some wheat lines have been modified by transgenesis for nutritional improvements (enhanced iron and oil/fatty acid content) [39] and by gene editing by Calyxt for fungal disease resistance (CRISPR-Cas) and high fiber content (TALEN). Both have a non-regulated status in the USA. The potential of genetic engineering of wheat is illustrated by the 81 patents collected here, with biotic or abiotic stress tolerance, weed control, nutritional properties, various agronomic or breeding-related traits.

It has recently been announced that the Argentine government has approved the drought-tolerant transgenic wheat variety HB4 which carries a sunflower gene, developed by Bioceres Trigall Genetics, a joint venture between Bioceres (Santa Fe, Argentina) and Florimond Desprez (Cappelle-en-Pévèle, France). Marketing will depend on export market acceptance. More generally, whether such traits can trigger renewed interest for biotechnological wheat and consumer acceptance, leading to commercialization remains to be seen. It should be recalled that glyphosate-resistant wheat developed by Monsanto was authorized for field tests in 16 states in the USA from 1998 to 2005 [40], but this wheat is not currently marketed because of potential market loss due to consumer reluctance [41].

Regarding biotechnological rice, only 5 events are approved/non-regulated in the USA: for bacterial disease resistance (TALEN; Iowa State University), bacterial blight resistance (CRISPR-Cas; University of Missouri), salinity tolerance (transgenesis using biolistics; Texas A&M University) and HT (ODM or gene editing *sensu lato*; Cibus) [42]. The compilation gathers 623 patents for rice with the following traits: abiotic stress tolerance (cold, drought, flooding, salt, cadmium, arsenic, diamide, iron-deficiency), biotic stress (striped rice borer, bacterial blight, blast, bacterial leaf streak, grey mold, rice stripe virus, weed control *via* glyphosate or glufosinate-ammonium tolerance), various agronomic features (yield improvement, grain weight and shape, root length, plant height, chlorophyll content and leaf senescence, flowering time, flower number) and breeding-related traits (improved transformation method, male sterility).

The impact of gene editing vs. transgenesis on biotechnological crops

The distribution of application sub-categories related to agronomy/nutrition is compared for transgenesis and gene editing in histograms 1 and 2 of Supplementary file 5. In the compiled ‘approved, non-regulated, marketed’ products, gene editing is used comparatively more for ‘other agronomic, harvest and post-harvest’ and for ‘nutritional’ applications, while transgenesis is used more for HT and resistance to biotic stresses. This trend is not mirrored in the patent landscape, but it should be borne in mind that the latter is heavily influenced by China and not the USA (see below).

Compared to transgenesis, for gene edited products the distribution of agronomy/nutrition traits per crop shows an increase for some species and a decrease for others (see Supplementary file 5, histograms 3 and 4). For the compiled ‘approved, non-regulated, marketed’ products, such an increase is seen for barley, citrus, rice, tomato and wheat, with a marked decrease for cotton, maize (two major transgenic crops) and sugarcane. A relatively lesser use of gene editing for cotton and maize, and for soybean, is also seen in the patent compilation. It can be concluded that the availability of plant gene editing techniques influences plant breeding at the level of both traits and crop species when compared to transgenesis, confirming what is apparent from the above discussion, namely that both techniques are complementary.

Plant produced biopharmaceuticals

Next to agronomic and nutritional traits, the interest of biopharmaceuticals made by plants should not be underestimated [43,44]. Using plants as biofactories could drastically reduce production time and hence product cost. In many cases, low temperature is not required for storage and transport. Regarding vaccines, edible vaccines may show advantages since no purification stage is required and the delivery is simplified. In addition, the risks of contamination by toxins or human pathogens are lower.

In this category, 25 patents are collected (Fig. 4a), all based on stable transgenesis. For approved/marketed products, 39 research projects which have reached at least the (pre)clinical stage were analyzed, all of which are based on classical transgenesis or transient expression after agroinfiltration. This also holds true for research projects at an earlier R&D stage (compiled in an appendix of the Vaccines/ Therapeutics page of Supplementary file 1). The prevalence of classical transgenesis may not appear surprising for biopharmaceutical production, which by nature involves the expression of a foreign peptide or protein. However, it is noteworthy that insertion of coding sequence *via* gene editing (also called SDN-3; [45]) is apparently not used.

In this study, the plant biotechnological approaches related to the Covid-19 pandemic have been collected as publicly available in March 2021. It is noteworthy that no such project has yet reached the market, which relativizes the potential utility of plant biotechnologies in this particular case. Four research projects have been announced as being at the (pre)clinical stage and 3 only at the R&D stage (see appendix of Supplementary file 1), but these statuses may change rapidly. In addition, one research project has already been officially discontinued [46].

Geographical discrepancies

Regarding plant biotechnological patents, China's leadership is hegemonic, as a likely convergence of multiple factors including massive investment in biotechnology, changes in patent laws, and also because patents are considered in this country as a tool not only to secure innovations but also for economic protectionism [32]. However, the overwhelming preponderance of China in the patent landscape contrasts strikingly with its current limited list of marketed products. The data compiled in Supplementary file 1 reveals 4 entries from China, all for biopharmaceuticals: 3 in rice (approved for pre-clinical trials: therapy of hypoalbuminia and Newcastle disease vaccine for poultry; at the R&D stage: a malaria vaccine) and one in tobacco (pre-clinical trials against COVID-19). While China holds 91 % of patents concerning rice, apparently no biotechnological rice for food has yet been brought onto the market [47]. The USA is the leader for actually marketed products or products that could be marketed (approved or non-regulated) with 76 % of the entries in Supplementary file 1 (89.5 % of products linked to Agronomy/Nutrition and 46 % in the case of industrial applications plus biopharmaceuticals).

A limited but growing number of African countries are taking the path of biotechnology in plant breeding [48]. Countries with commercialized transgenic crops and on-going field trials are Sudan, Ethiopia, Kenya, Malawi, Nigeria, Eswatini (formerly Swaziland) and South Africa. Countries with on-going trials but no commercial cultivation are Uganda, Tanzania, Mozambique, Burkina Faso and Ghana. These African field trials are usually supported by private foundations, the three largest being the Bill and Melinda Gates foundation, the Howard Buffett foundation and the Rockefeller foundation. Each research project is devoted to a specific crop. Several field trials are often conducted in different membership countries.

The broader 'HarvestPlus' research project/program [49], concentrating on the delivery and scaling up of biofortified crops in Africa, India and Latin America, is performing field trials of crops such as cassava, rice and sorghum, focusing on zinc, iron and vitamin A deficiencies.

Regarding agronomic plants with traits to overcome biotic stresses, consortia of universities, national institutions, foundations and seed industries in Africa are focussing on banana, cassava, maize, potato, and sweet potato to address specific African challenges. For abiotic stresses, trials testing plants for resistance to freezing, heat or mineral deficiencies (phosphate, nitrogen, iron or sulphur) are noteworthy.

In terms of event approvals and commercialization of biotechnological varieties, South Africa ranks first with approval of transgenic canola, cotton, maize, rice and soybean. It is among the world's top 10 countries planting biotechnological crops (2.7 million hectares in 2019). The country is also performing research on new traits, such as insect resistance stacked with moderate drought tolerance in maize (TELA research project/program; [50]), stacked traits with modified fatty acid composition in soybean, insect resistance and herbicide tolerance multi-stacks in cotton, insect resistance in potato, sugarcane and wheat. Sudan comes next with 0.2 million hectares in 2019, namely Bollgard™ and Ingard™ Bt cotton resistant to Lepidopteran pest insect, developed by Monsanto [51]. No evidence is apparently available indicating that this country is performing innovative biotech field trials. Eswatini has also introduced a lepidopteran insect-resistant transgenic cotton [52].

Nigeria has approved transgenic cotton, cowpea, maize and soybean although, to date, only transgenic cotton and cowpea are actually cultivated. Concerning other innovative traits, research is being conducted on cowpea for insect resistance to Maruca pod borer in cowpea, pro-vitamin A biofortification in sorghum (ABS research project), 'Nitrogen Use, Water Efficiency and Salt Tolerance' (NEWEST research project) in rice, delayed postharvest starch deterioration in cassava and on the TELA research project in maize [53].

Kenya introduced Bt cotton in 2020 and research includes TELA, ABS, Cassava Brown Streak Disease (CBSD) resistance and insect resistance [48]. In 2015, Burkina Faso abandoned its only biotech crop cultivated, Bt cotton, but research is ongoing including insect resistance to *Maruca* pest in cowpea. In 2018, Ethiopia approved its first transgenic crop, a Bt cotton, which is not yet cultivated. Research is part of the maize TELA research project [48].

Research is conducted in Uganda on the TELA and NEWEST research projects, on banana Xanthomonas wilt resistance, parasitic nematode resistance, biofortication in banana, on CBSD resistance and Cassava mosaic virus resistance (also in Kenya; both using RNAi), and Late Blight disease resistance in potato [48]. Cultivation of a transgenic banana resistant to *Fusarium oxysporum* and to Banana bunchy top virus (BBTV) in field trials in Uganda (and Australia) is also noteworthy [48,54]. A transgenic banana tolerant to drought has been tested in Uganda (Banana21 research project [55]).

Other research projects also include: in Malawi, insect resistance in cotton and cowpea, BBTV resistance in banana and plantain and the Banana21 research project; in Ghana, the NEWEST project and insect resistance to Maruca pod borer in cowpea; in Mozambique and Tanzania, the TELA research project. Finally, maize varieties tolerant to drought are being tested in Ethiopia, Kenya, Mozambique, South Africa, Tanzania and Uganda (WEMA, water efficient maize for Africa [56].

In regard to the EU, the data confirm that regulations have discouraged many developers, including the public sector, and this includes field trials [57]. While there was hope that simple mutations obtained *via* gene editing would not be considered as GMOs [58], the path the EU seems to be taking [59] is more likely to impede the marketing of many of the useful advances highlighted here, each time the European market is concerned. The EU regulatory context on technologies has downstream consequences in approval processes and related political economy. As pointed out by many economists and political scientists, investment in new applications is related to expected economic benefits, which in turn are affected by the regulatory environment. The latter may increase or decrease the costs for developers of technologies, here plant breeders, a phenomenon which has been widely discussed [60]. It remains to be analyzed in detail what the upstream reasons are for choosing a favorable or an inhibitory regulation depending on the countries. Why the EU was at the origin of such restrictive policies on biotechnologies (starting with the 1990 'GMO' Directive [61] and consistently amplified afterwards) and not the USA for example, has been linked to the 20th century history of both regions, which created *inter alia* contrasted policies on technological risks [62].

Public and private sectors

Regarding the leaders in development of biotechnological plants, the distribution of recently approved/marketed products is: BASF (Ludwigshafen, Germany; Florham Park, NJ, USA; 3 transgenic), Bayer/Monsanto (21 transgenic, 1 together with BASF), Calyxt (6 gene edited), Ceres (5 transgenic), and Corteva (5 gene edited) with three new companies CIBUS (14 gene edited), J. R. Simplot Plant Sciences (9 gene edited, 6 transgenic) and Living carbon PBC (San Francisco, CA, USA; 8 transgenic). The patent distribution for these companies is: BASF (3 transgenic) and Bayer/Monsanto (3 transgenic, 1 gene edited).

Out of the 259 patents involving a CRISPR-based gene editing, only 28 were filed by private companies, and only one of which by the above-mentioned major companies. The other private companies are from China (18 companies), USA (4), Switzerland (2), UK (1) and Sweden (1). Patents from the public sector are mainly from China.

Conclusions

Biotechnology uses are expanding to a wider range of plants to address diverse issues in agriculture for both food and non-food purposes, such as therapeutic or industrial applications (some of which have been commercialized). Currently, gene editing techniques appear to be an efficient complement to classical transgenesis rather than a replacement. However, a steep increase is observed in gene-edited products in 2020 (at least in non-regulated products in the USA). Smaller companies and academic laboratories hold a significant share of gene editing patents. However, it remains to be seen how many products will reach the market and what the worldwide impact of EU regulation of gene-edited plants will be. While China is an overwhelming leader in plant biotechnological patenting, the country is still far behind the USA in the marketing of such products.

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Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.nbt.2021.09.001.

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