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The contribution of transgenic plants for addressing global challenges through modern plant breeding techniques

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Abstract

This survey's objective is to identify and describe new plant biotechnology products that have come onto the market since 2010, with a focus on those that are related to the development of New Breeding Techniques (NBTs), such as gene editing using the CRISPR-Cas system. We compile information on transgenic (gene transfer or gene silencing) and gene edited features that have received approval or are commercialised in at least one nation, or that are not subject to regulation in India. We also compile information on related patents from around the world. Also looked at are field trials conducted across the continent to offer insight on prospective innovations for India. Agronomic advancements, industrial use, and medical use specifically, the synthesis of recombinant medicinal molecules are all classed within the application categories in which the data have been assembled. According to the statistics, gene editing does not appear to be a replacement for 'classical' transgenesis, the usage of which is increasing, but rather a useful addition to it. This is a tendency that has also been seen in the patenting environment. However, it is clear that gene editing is being used more frequently. In comparison to transgenesis, gene editing has resulted in a rise in some crop species and a decrease in others among crops that have been approved, unregulated, or commercialised. Breeding traits show a similar divergent tendency. New private businesses have benefited from gene editing as well. The patenting landscape is overwhelmingly dominated by China, particularly by its governmental sector, as opposed to the approved/marketed landscape, which is dominated by the USA. The statistics suggest that regulatory frameworks will encourage or stifle innovation.

Keywords: Transgenic plants, plant breeding, biotic stress, biotechnology

Introduction

Since the 1980s, the development of gene transfer technologies (transgenesis) has greatly aided basic and applied research, and some of its products have been put on the market since the middle of the 1990s [1]. Here, these methods are referred to as "classical." The use of sequence-specific nucleases for gene editing in plants, including TALENs (Transcription Activator-Like Effector Nucleases), CRISPR-Cas systems (Clustered Regularly Interspaced Short Palindromic Repeats), and Oligonucleotide-Directed Mutagenesis (ODM) technologies, could be the next great advance [2]. Recent publications [3–9] provide overviews of the application of CRISPR-based gene editing in plants, as well as its difficulties and future opportunities. A report by German scientific authorities [10] and an essay by Purnhagen and Wesseler [11] also highlighted a variety of applications and possible applications of these technologies while examining the legal situation and implications of gene editing in the EU.

The recent breakthroughs in plant biotechnology that have been approved or sold have been assembled in the current review, which adopts a new perspective, and traditional transgenesis and gene editing are set apart. Gene transfer is distinct from gene silencing by anti-sense or RNA interference techniques, generally known as RNAi, with regard to classical transgenesis [12]. Additionally, patents utilising conventional transgenesis or the CRISPR-Cas system in plants have been collated in order to identify the most recent developments. The focus is on Africa because, according to the International Service for the Acquisition of Agri-biotech Applications (ISAAA), it has "the biggest potential to reap benefits associated with modern agronomic biotechnology" [13]. Examining field trials may also shed light on original research projects. The objectives are to examine whether gene editing involves new plants, new traits, or new actors, whether traditional transgenesis techniques and gene editing are complementary or rival, and how biotechnology might offer tools to address global challenges in agriculture.

Materials and Methods

Approved, non-regulated and marketed biotechnological plants

The ISAAA GM database was used to construct a list of biotechnological plant types that have been given the go-ahead for commercial usage by regulatory bodies worldwide or that have already been commercialised in at least one nation [14]. This includes varieties that underwent risk assessment and were granted 'deregulated' status in the USA [15]. Additionally, the 'Am I regulated' US Department of Agriculture - Animal and Plant Health Inspection Service (USDA-APHIS) database in the USA was used to find items that were exempt from restrictions (i.e., non-regulated) [16]. Another information source that is used is the websites of potential developers. "Other gene editing (not disclosed)" was tagged on the data when "Corporate Business Information" (CBI) was not accessible. Additionally, plant lines expressing 'novel proteins' that are not pesticides that have been examined for food safety by the US Food and Drug Administration (FDA) were obtained from [17]. The compilation of the Approved, Non-Regulated or Marketed New Biotechnological Plants portion of Results excludes plants in the Research and Development (R&D) stage.

Plant biotechnology patents

The Orbit Intelligence database was used to find patents for innovations based on the CRISPR-Cas technology or traditional transgenesis (gene transfer or RNAi) [18]. The patent supplemental file includes a diagram of the search query equation. The 45 significant species gathered in the "approved and marketed" section were the only ones included in this search. Patent titles, abstracts, inventors, applicants, priority dates, and various reference numbers were reorganised into patent families, which include all extensions of a single invention.

Manual sorting

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

Results

Approved, non-regulated or marketed new biotechnological plants

The compilation, which spans January 2015 to October 2020, is presented in two datasheets of Supplementary File 1, with a total of 219 entries (including 152 related to agriculture, 20 to industrial use, of which 2 are stacked (i.e., genetic traits grouped in a single variety; also known as pyramided) with an improved agronomic trait), 39 to therapeutic use, and 10 non-available data (annotated as 'nd', not disclosed). Some entries are made up of stacked individual occurrences, most frequently herbicide tolerance with another characteristic. Additionally, some singular events contain several traits. All stacking events and the features mixed in some events were individualised in order to conduct a more in-depth and quantitative study. Redundant qualities were only counted once in order to create a relevant overview of the fraction of each technique creating a total of 222 individualised non-redundant traits. The sections that follow analyse this unique dataset. Herbicide tolerance, biotic stress, abiotic stress, or other agronomic, harvest and post-harvest features, as well as nutritional traits were divided into subcategories of agronomic traits.

Herbicide-tolerant (HT) biotechnological plants

Herbicide resistant characteristics, which account for 24% (40 traits) of the improved agronomic/nutritional features, are primarily made for two herbicides: glufosinate (LibertyLink technology of Bayer, Leverkusen, Germany) and glyphosate (Roundup-Ready technology of Monsanto, Saint Louis, MO, USA). The market currently offers transgenic carnations resistant to sulfonylurea herbicide, cotton resistant to Oxynil, maize or soybean resistant to 2-4 D, and Dicamba. Most of these traits are transgenic. A rice and flax HT variety (CBI not given) that Cibus (San Diego, CA, USA) obtained utilising ODM technology has been granted non-regulated status in the USA. Due of the rising stacking of both trait categories, along with biotic stress resistance traits.

Biotechnological plants with biotic stress resistance traits

Pests such insects, nematodes, tiny fungus, bacteria, and viruses generate biotic stressors, which make up 19% (31 attributes) of enhanced agronomic/nutritional aspects. These features' frequency is depicted in comparison to the more common HT traits.

With the exception of meganucleases, all techniques are represented, including gene silencing by RNAi (2), gene editing (7), five of which use CRISPR-Cas9, one TALEN, and one whose technical details are not disclosed. Gene transfer by classical transgenesis, which involves 19 traits transferred via Agrobacterium tumefaciens and four by biolistics. There are 12 different plant species. These transgenic traits are currently available in cotton, cowpea, maize, and soybean, and sugarcane traits are approved for cultivation (in at least one nation). In at least one nation, transgenic potato, rice, sugarcane, and tomato lines have been given the go-ahead for planting. While transgenic varieties are commercialised, gene editing did not result in goods that were introduced to the market in this pest resistance subgroup between 2015 and 2020 (some have a non-regulated status that makes them marketable).

InnateTM potatoes, which are sold by J.R. Simplot Company (Boise, ID, USA), are resistant to the fungus Phytophthora infestans and are a good example of bacterial, viral, or fungal disease resistance. At least one nation has approved the cultivation of canola varieties obtained by Cibus via gene editing for fungal disease resistance, citrus varieties obtained by Soilcea (Tampa, FL, USA) via CRISPR-Cas for viral disease resistance, maize varieties obtained by DuPont Pioneer (Johnston, IA, USA) via CRISPR-Cas for fungal resistance, and wheat varieties obtained by Calyxt (Roseville, MN, USA) via CRISPR-C. In the USA, soybean lines developed by Evogene (Rehovot, Israel) utilising CRISPR-Cas have been deregulated due to their improved resistance to soybean cyst nematode.

Biotechnological plants resistant to abiotic stresses

5.5% (9 attributes) of the improved agronomic/nutritional qualities fall within this area. Four species have developed tolerance to drought and/or salinity, which are relevant abiotic stressors. Transgenesis (4 via gene transfer via Agrobacterium and 2 by biolistics) and CRISPR-Cas9 (3) are the methods used. of Supplementary file 2 contains a summary of the data. One maize line was obtained by CORTEVA (Wilmington, DE, USA) and one by Monsanto, one soybean line by Indear (Rosario, Argentina), one by USDA ARS (St. Paul, MN, USA) and one by Verdeca, a partnership between Arcadia Biosciences (Davies, CA, USA) and Bioceres Crop Solutions Corp. (Santa Fe, Argentina), one rice line by Texas A&M

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

For crop productivity or harvest/post-harvest quality, this subcategory covers a number of enhanced aspects of direct significance to farmers. Of the total number of enhanced agronomic/nutritional traits, it accounts for 32% (53) (52). The production of biomass prior to harvest (increased yield) or pollination control (male sterility, fertility restoration) are two examples of traits that are relevant. A third pertains to post-harvest characteristics (shelf-life, non-browning, reduced black spot formation after bruising, delayed fruit softening), while a second relates to harvest stage (delayed ripening/senescence, flower or fruit colour). RNAi gene silencing (7), gene editing (CRISPR-Cas9: 27 characteristics; TALEN: 2; meganuclease: 1; unknown details: 2), or traditional gene transfer were used to acquire these traits. The Supplementary file 2's lists the 23 pertinent species. Developers are mostly from the USA, Germany, Japan, USA + Canada, and Israel, in that order. It should be noted that the transgenic chrysanthemum variety was acquired by Suntory Flowers Limited (Tokyo, Japan) and that importation into the USA is unrestricted.

Biotechnological plants with nutritional improvements

Of all the enhanced agronomic/nutritional traits, this subcategory accounts for 19% (32). Phytase production, reduced asparagine and reducing sugar content to reduce production of acrylamide upon frying, or modifications in oil/fatty acid, carbohydrate, lignin, protein or vitamin A content are some examples of nutritional improvements for humans or animals) [19]. These characteristics were either acquired through traditional transgenesis, such as gene transfer (11 features via Agrobacterium) or RNAi gene silencing (7), or through gene editing techniques including TALEN (5), CRISPR-Cas (9), meganuclease (1), and hidden 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).

In addition to other features, RNAi was employed to create potato lines with a reduced potential for acrylamide generation during cooking. The latter characteristic will also lessen post-frying browning and the development of bitter flavours. The several potato cultivars sold under the generic name "InnateTM" (see above) share the same gene construct that was introduced in different genetic backgrounds or the same gene construct that was inserted in distinct events in an Elite genetic background.

One alfalfa line with low lignin content, two soybean lines with modified oil/fatty acid content, and one wheat line with high fibre content were all developed using TALEN technology. Meganuclease was employed in maize for the processing and animal nutrition sectors. Pennycress lines with altered oil/fatty acid content, soybean lines with altered seed composition, wheat and tobacco lines with unknown applications, canola lines with altered oil content, pea lines with improved flavour, three potato lines (2 with reduced glycoalkaloids, 1 with reduced vacuolar invertase which affects reducing sugar content), and soybean lines with high oleic acid content were all produced using CRISPR-Cas9.

Geographical distribution of plant biotechnology patents

With 1564 patent families, or 90% of the total, China takes the top spot. The USA falls far behind with only 3.7% (65 patent families), while being the leader in plant biotechnology sales. Korea, which imports transgenics but does not permit their growing, reported 3.2% (56). Only 1.2% of the total population are EU Member States (21). The patents that were publicly accessible on June 16, 2020, when the database was last screened, are represented by these data. It was required to consider that China publishes a large number of patents before the delay of 18 months following the initial priority date in order to more appropriately assess the relative patenting weight of each nation [20]. This 18-month gap before the most recent database update corresponds to the 16th of December 2018 thus. In fact, 407 patents were published after December 16th, 402 of which were filed by China. The proportion between China (87.4% of submitted patents) and other countries (USA: 4.7%, Korea: 4.1%, and Europe: 1.5%) does not change considerably when they are excluded, though.

Patent landscape according to biotechnology method

With 75% of all patents combined, conventional transgenesis is still the most popular method for transferring genes. In comparison to RNAi, CRISPR-Cas9 was employed in 14% of these patents. China (92.5%, 235 patent families) has filed the majority of the CRISPR patents, followed by the United States (4%, 10 families), Europe (2.4%, 6 families), Saudi Arabia (0.8%, 2 families), and Korea (0.4%, 1 family). China dominates in all technological fields. Below is a discussion of how patents have changed throughout time according to technique.

Distribution of biotechnology patents by category

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%) were the four categories into which patents were manually sorted to further this analysis. Technical improvement was the fourth category, which included transgenesis or gene editing, novel promoters, or screening techniques (16%).

Discussion

The proportion of various technologies

222 unique non-redundant traits were sorted in 45 varieties to find recent trends in plant biotechnology using a database of biotechnological events that have been approved or marketed in at least one nation. Of these traits, 70% were obtained using traditional transgenesis for gene transfer or silencing (RNAi, in 8%). Surprisingly, despite the fact that gene editing is frequently regarded as a fresh biotechnological revolution, the current compilation only identifies 29.7% of individualised traits obtained by gene editing techniques: 21.6% by CRISPR-Cas, 3.1% by TALEN, 1.4% by

Meganuclease, 0.9% by ODM, and 2.7% by unreported gene editing techniques. the breakdown of methods by species.

The cases of major crops

Whether new advancements (through traditional transgenesis or contemporary gene editing approaches) will permit diversity of biotechnology crops, which frequently involves overcoming regulatory and financial limits, is a crucial topic. It's remarkable that there is now no biotechnological wheat available on the market for wheat, which is the second most produced crop after maize and before rice [21]. Some wheat lines have been altered by transgenesis for nutritional improvements (enhanced iron and oil/fatty acid content) [22] and by gene editing by Calyxt for fungal disease resistance (CRISPR-Cas) and high fibre content (TALEN), despite their relative resistance to *in vitro* culture and regeneration. Both are legal in the USA but not regulated. The 81 patents included here, with varied agronomic or breeding-related features, biotic or abiotic stress tolerance, weed management, or nutritional properties, demonstrate the promise of wheat genetic engineering.

The drought-tolerant transgenic wheat variety HB4 with a sunflower gene, created by Bioceres Trigall Genetics, a partnership between Bioceres (Santa Fe, Argentina) and Florimond Desprez (Cappelle-en-P'ev'ele, France), has recently been announced as having received approval from the Argentine government. Marketing will be based on how well export markets perform. More generally, it is unknown if these features will rekindle consumer interest in biotechnology wheat and promote its commercialization. Recall that Monsanto's glyphosate-resistant wheat was authorised for field trials in 16 US states from 1998 to 2005 [23]. However, this wheat is not currently being sold due to probable market loss from consumer resistance [24].

In the USA, only five biotechnological rice events have been approved or are unregulated: 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) [25]. The compilation gathers 623 patents for rice with the following traits: biotic stress (weed control with glyphosate or glufosinate-ammonium tolerance), abiotic stress tolerance (cold, drought, flooding, salt, cadmium, arsenic, diamide, iron-deficiency), and various agronomic features (yield improvement, grain weight and shape, root length, plant height, chlorophyll content, and lean).

The impact of gene editing vs. transgenesis on biotechnological crops

In histograms 1 and 2 of Supplementary file 5, the distribution of agronomy/nutrition-related application subcategories for transgenesis and gene editing are compared. Gene editing is employed comparably more for "other agronomic, harvest and post-harvest" and for "nutritional" applications in the gathered "approved, nonregulated, marketed" products than transgenesis is for HT and tolerance to biotic stressors. Although the patent landscape does not reflect this trend, it is important to keep in mind that China, not the USA, has a significant influence on it.

When compared to transgenesis, the distribution of agronomy/nutrition attributes by crop increases for some species while decreasing for others in gene altered products. Barley, citrus, rice, tomatoes, and wheat are among the 'licenced, non-regulated, marketed' items that have seen an increase, while cotton, maize (two of the main transgenic crops), and sugarcane have witnessed significant decreases. The patent compilation also reveals a comparatively reduced use of gene editing for cotton, maize, and soybean. When contrasted to transgenesis, it can be said that the availability of plant gene editing techniques has an impact on plant breeding at the level of both characteristics and crop species, proving that both methods are complementary.

Public and private sectors

Regarding the companies that have been at the forefront of the development of biotechnological plants, the distribution of recently approved/marketed products is as follows: BASF (Ludwigshafen, Germany; Florham Park, NJ, USA; 3 transgenic), Bayer/Monsanto (21 transgenic, 1 in collaboration with BASF), Calyxt (6 gene edited), Ceres (5 transgenic), and Corteva (5 gene edited), with three new companies CIBUS (14 gene edited), J. These businesses' patent distribution is as follows: Bayer/Monsanto (3 transgenic, 1 gene modified) and BASF (3 transgenic). Only 28 of the 259 patents incorporating CRISPR-based gene editing were submitted by private businesses, and only one of those was done so by the two major corporations mentioned above. The remaining private businesses are from Sweden (1), Switzerland (2), the United Kingdom (1), America (4), and China (18 enterprises). The majority of public sector patents come from China.

Conclusions

In order to solve a variety of difficulties in agriculture for both food and non-food purposes, such as therapeutic or industrial applications (some of which have been commercialised), biotechnology uses are expanding to a larger range of plants. At the moment, gene editing methods seem to be an effective addition to traditional transgenesis rather than a replacement. At least for unregulated products in the USA, a sharp increase in gene-edited products is predicted in 2020. A sizable portion of gene editing patents are held by smaller businesses and academic research facilities. It is yet to be determined how many goods will be available on the market and what effect the EU's regulation of gene-edited plants will have globally. Although China dominates the patenting of plant biotechnologies, the nation is still far behind the USA in the marketing of such goods.

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