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Role of genome editing of plants by CRISPR/Cas9 for virus resistance: Patent analytics

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Abstract
CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a trendsetting technique that is used for gene editing. Molecular life sciences have been revolutionized by this. This work categorizes a range of CRISPR-related patents and their application to crop improvement. The patents are classified into groups according to the type of plant CRISPR vectors, gene targeting and genetic modification of plants, approaches to produce non-transgenic, genome editing in tomato, potato, rice and other crops, gene targeting in plants using DNA viruses and genome editing for virus resistance plants appearing in the claims. These recent advances in CRISPR/Cas, gene editing techniques have made cell engineering easy and cheaper, propelling its demand. The goal of the applications is to increase resistance to abiotic or biotic stress, to engineer metabolic pathways, and to increase grain yield. This patent documents and applications for CRISPR are known as to be state-of-the-art technological information sources. Patent analysis allows scientists to obtain valuable knowledge that is often not revealed in published scientific literature. The patent field of CRISPR-Cas9 is dominated by industrial firms from universities and research institutes. The global trend suggests that no CRISPR patent is accessible from the Indian subcontinent for crop improvement. In examining the trend over a period of time, the study takes into account the important changes that have taken place and have direct bearing on the patenting activity. The aim of the summary is to facilitate the adoption of CRISPR for the importation of crops and the production of patentable technology.

Keywords: CRISPR/Cas9, virus resistance, vector systems, patent analysis

Introduction
It is anticipated that the global food system will provide safe and nutritious food to a population that is likely to grow from 7.5 billion people today to almost 10 billion by 2050. Food production however is only one aspect of the food system. The agro-food sector is also providing millions of people with a livelihood. Globally, most people living in extreme poverty live in rural areas where the most important economic activity is often food production. A significant environmental impact is still present in the global food system. Agriculture currently covers about 40 percent of the surface of the earth, even more than any other human occupation. In addition, agricultural crop irrigation accounts for 70 percent of global water use and agriculture contributes directly to around 11 percent of global greenhouse gas (GHG) emissions (mostly through cattle). Deforestation, additional GHG pollution, and the depletion of biodiversity will also result from expanding agricultural land.

India is faced with the twin problems of a growing population and the need to increase the production of food without damaging its quality Systems of Food. These problems will have to be tackled. Usage of our restricted capital. Production in agriculture requires Land, water and energy resources; therefore the sustainable use of land, water and energy resources the need for the hour is to help with emerging developments.

Next Generation Sequencing technology has made it easy to discover and detect virus variability and computational biology also helped to understand plant-virus interactions to identify potential targets for virus resistant plant development (Hadidi *et al.*, 2016) [9]. But the durability of the virus resistance in plants is still the major drawback due to the diversity and rapid virus evolution. Developing plants with resistance to viruses is considered to be the most comprehensive approach to monitor the rapid development of plant viruses for economic and environmental prosperity. A promising tool for plant genome engineering has emerged the CRISPR (clustered regularly interspaced palindromic repeats)/CRISPR-associated system 9 (CRISPR/Cas9) (Jinek *et al.*, 2012, Mali *et al.*, 2013) [14, 23]. It has become a simple, most users friendly and effective, accurate genome editing tool for genetically modified crop development (Khatodia *et al.*, 2016, Schiml *et al.*, 2016, Zaidi *et al.*, 2016) [15, 16, 34, 42]. It has become the most promising and extremely versatile crop improvement tool for providing sustainable

productive agriculture in a changing climate for better feeding of rapidly growing populations (Khatodia *et al.*, 2017) [15, 16]. The CRISPR/Cas9 system is an RNA-based programmable endonuclease-based technology consisting of 2 components, the Cas9 nuclease and an engineered RNA guide that targets any DNA sequence of the N20-NGG form used in many organisms including plants for novel genome editing applications (Jinek *et al.*, 2012. Khatodia *et al.*, 2016, Schiml *et al.*, 2016, Zaidi *et al.*, 2016) [14-16, 34, 42]. Recently many studies reported that CRISPR/Cas9 mediated the development of virus resistance in plants with promising durability of resistance (Khatodia *et al.*, 2017) [15, 16]. Delivery of the pre-assembled Cas9/gRNA ribonucleoprotein complex for virus resistance development in any plant species as a novel DNA-free genome editing technique was envisaged.

Plant pathogens, are major yield-limiting factors. That cause global agricultural productivity losses of 20-40 percent, posing significant challenges to food safety and security and thus remains a major global agricultural challenge (Savary *et al.*, 2012; Rodriguez-Moreno *et al.*, 2017, Mushtaq *et al.*, 2019) [24, 29, 33]. During the last decade, the concept of Genome editing (GE)/crop plant modification revolutionized all aspects of plant science. To date, four different site-specific endonuclease-based systems, namely CRISPR/CRISPR associated protein 9 (CRISPR/Cas9), zinc-finger nucleases (ZNFs), transcription-like effector nucleases (TALENs) and mega nucleases (Osakabe *et al.*, 2010; Baltes *et al.*, 2015; Zaidi *et al.*, 2016; Mushtaq *et al.*, 2018) [24, 26, 42] have been widely used. Our rationale for CRISPR/Cas9 genome editing to virus resistance generation, which can be applied directly to important crops in the future. Given that CRISPR/Cas9 is a viable technology for site-specific genome editing in several plant species, we believe this work will pave the way as a strategy for reverse engineering virus resistance in a wide variety of crops.

CRISPR patent analytics

Eighty percent of the science and technological information uncovered in the released patents is projected not to be reported elsewhere. There are practically tens of millions of registered patents and references to patent applications available to the public for analysis. This overwhelming treasure chest of data can be made available only by identifying the critical, appropriate references in a given technology and then updating those references in a way that provides evidence for actionable decision-making. In recent years, patent research has grown in significance. It is now used as a distinctive management instrument for discussing a technology's competitive management. Patents are considered as the earliest source of disclosures for new technologies. By assessing patenting trends, we can identify key technology areas, major players in the field, and current focus of research. This research is particularly useful for input-intensive, evolving and frontier technologies such as agri-biotechnology. Patents, regardless of their grant status and market merit, are a consequence of R and D operation, according to Ashton and Sen (1988) [1] and Ernst (2001) [7]. Patent statistics thus provide historical insights that can approximate or predict future technology changes. Patent research also provides general developments and prospects for leading actors in the business domain in particular technology fields, technological competition, and R&D strategic directions (Park *et al.*, 2013) [27]. A completed project for patent analysis consists of a collection of technical sources and associated analytics from which it is possible to derive valuable legal, market, and technology knowledge. This data helps big businesses, start-ups, universities, academic organizations and analysts to consider and make educated choices before spending time and resources in emerging innovations and prospects for product growth.

Methodology for the study

Generating new plants with enhanced or attractive characteristics has historically relied on laborious and time-consuming breeding techniques. Farm production with enhanced characteristics fuels the existing dependency on plant resources of agriculture and different industries. A modern age in genetic engineering has culminated in genome-editing technology, enabling effective, reliable and rapid engineering of the genomes of plants. A modern gene-editing genome has appeared as a cluster of frequently interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (CRISPR/Cas9). The instrument has been commonly used in numerous animals, including plants. The use of CRISPR/Cas9 enables the production of null sergeants (transgene-free genome-edited plants) within a limited period of time. Our study focuses on exploring emerging trends in CRISPR biotechnology applications for crops. More precisely, we used patent analytics to gauge patterns in the growth of crop-resistant virus disease traits. This report also try to highlight the important technological directions and gaps in our knowledge in order to allow further pursue of R&D, using data from different databases, namely European Patent and trade mark office database (EPO), Google patents, Indian patent database (IPO), United States Patent office database (USPTO), World Intellectual Property Organization (WIPO), China National Intellectual Property Administration (CN), Japan Patent Office (JP), Canadian Intellectual Property Office, Korea Intellectual Property right information, IP Australia, German Patent and Trade Mark Office, Israel Patent Office and Intellectual Property Office of Singapore (IPOS). From 2004 to 2020, all searches and data are collected to cover successful patenting agencies around the world. The following key words were used in the international search for patents on a particular subject: RNA guided genome editing, patents for plant CRISPR vectors, gene targeting and genetic modification of plants, non-transgenic production approaches, genetically modified plants or crops, genome editing in tomatoes, potatoes, etc., gene targeting in plants using DNA viruses, genome editing in plants with virus resistance. The WIPO, USPTO, EPO, PCI, JPO, KIPO, INPADOC, IPO and all electronic databases were searched using the advanced Boolean search on the date of issue, the country and international classification number and the bibliographic references of all patents. This was done in order to understand the technical approaches taken throughout the world by various research groups. It also provided an insight into emerging technologies and key areas for research and development. Our approach involves identifying key players within the sector and mapping the relevant technologies they have developed. As a primary source of information, this data can be used to further identify other players (those not listed among the key players) and identify the technologies in which they are involved. Under the platform technology of CRISPR virus resistant trait development in crops, this iterative process would help identify major, minor or unexplored technologies. Using "Acclaim IP," a patent search and analytics service (http://www.acclaimip.com/), we have developed an overview of the CRISPR tool application for crop improvement.

Clean-up entity

Assignee names appearing on patent papers are sometimes pronounced and/or formatted inconsistently. These were to the maximum practicable, regularized.

Categorizing

According to a taxonomy created by the authors, patent documents were manually placed into technology divisions, using the details found in patent names, abstracts, claims or technology classification codes.

Results and Discussion

The 'agricultural imperative' has been appreciated internationally, where food supply needs to be doubled by 2050, with lower resources and smaller carbon footprints, to feed the estimated nine billion populations. Also it is a Key to Sustainable Targets for Sustainability. Emmanuelle Charpentier, of the Max Planck Unit for the Study of Pathogens, Berlin, Germany, and Jennifer A Doudna, of the University of California, Berkeley, USA, was awarded the Nobel Prize in Chemistry 2020 for the gene editing technique known as CRISPR/Cas9 scissors. No wonder, for medicinal and biotechnological uses, this breakthrough has opened up new horizons. This mechanism is being examined in different bacteria present in diverse conditions, such as hot springs, human body, soil, etc. This nano-machinery has been wonderfully harnessed to rewrite various genomes across different kingdoms since the discovery of the basic mechanism of CRISPR-Cas action in Streptococcus pyogenes. With the help of inactivated viruses, the machinery is directed at the cells. The machinery is now used to cure/resist persistent pathogenic viral diseases with the aid of viruses, originally known to wade out of viruses. Oh what a clever way! The global market size for genome editing/genome modification is expected to hit USD 11.2 billion in 2025, compared to USD 5.1 billion in 2020, at a CAGR of 17.0% over the forecast period. Factors such as the availability of government funds and increases in the number of genomics projects are primarily driving market growth. The high cost of genomic equipment, however will hold back the development of this industry.

In newer fields of animal and disease model advancement, drug compounds and target screening and agricultural and nutritional health goods, the promise of technology has been explored, as research and development investment in technology is rising by leaps and bounds. In agriculture, CRISPR-Cas9 instruments can play a multi-faceted function by allowing the seed editing industry to make them more nutritious, enhanced flavour, disease tolerant and less vulnerable to drought. The technology section of agriculture and plant breeding is constrained in development by many possible regulatory criteria and ethical apprehensions. The present study data was examined with respect to (i) trends in the growth of patenting activity (ii) organizations/ industries active in research, and (iii) the focus of research pertaining to present situation. By highlighting the major technical developments and differences, the study was used to suggest evolving technological possibilities and developments. Here is the gist of the conclusions derived from the study.

Engineering plant genomes using CRISPR/Cas systems

First patent on CRISPR identified, US7919277 Detection and typing of bacterial strains, held by Danisco and invented by Barrango revolutionized the CRISPR–Cas scientific and regulatory activity. Genome editing (GE) has modernized the biological world by providing a means to edit genomes of living organisms, including humans, plants, animals, and microbes. Until now the CRISPR/Cas9 method has been the best option for genome editing (GE), but considering its broad use and implementations, there are still certain drawbacks to its more mainstream use.

The CRISPR/Cas9 technology has revolutionized genome engineering to include simple, accurate and inexpensive site-specific genetic manipulation and control tools (Doudna and Charpentier, 2014; Hsu, Lander, and Zhang, 2014) [5, 11, 14, 28, 43]. The method has been successfully used in a number of primary cells, cell lines and species since its discovery in 2012, and nearly all model systems. CRISPR/Cas9 is a derivative of type II CRISPRR, a bacterial adaptive immune system (Chylinski, Makarova, Charpentier, and Koonin, 2014) [5, 14]. Cas9 is a dual endonuclease capable of causing complex double stranded DNA breaks in natural and engineered structures (Jinek *et al.*, 2012) [14]. The targeting precision is provided by short Cas9-bound RNA(s) composed of Cas9-binding scaffold and 20 nt adjustable base-pairing guiding sequence with a desired target DNA sequence. Several study groups have researched and upgraded the device in recent years by adjusting scaffolds for greater performance and proposing recommendations for target site selection to include more precise and active cleavage (Hsu *et al.*, 2014) [11].

This tool features a procedure for altering the genetic material in a plant cell. The approach may involve (a) inserting into the cell a nucleic acid comprising a crRNA and a tracrRNA, or a chimeric critracrRNA hybrid, wherein the crRNA and tracrRNA, or the cr/tracrRNA hybrid, is targeted to a sequence that is endogenous to the plant cell; and (b) a Cas9 endonuclease molecule is inserted into the cell that causes a double strand break at or near the sequence targeted by the crRNA and tracrRNA sequence, or at or near the sequence targeted by the cr/tracrRNA combination. The Cas9 endonuclease and the crRNA and tracrRNA, or the tracrRNA hybrid, can be delivered to the plant cell by a DNA virus (e.g., a geminivirus) or an RNA virus (e.g., a tobravirus). The sequences encoding the Cas9 endonuclease and the crRNA and tracrRNA or the critracr RNA can be delivered to the plant cell in a T-DNA, with the delivery being *via* Agrobacterium or Ensifer. The sequence encoding the Cas9 endonuclease can be operably linked to a promoter that is constitutive, cell specific, inducible, or activated by alternative splicing of a Suicide exon. The plant can be monocotyledonous (e.g., wheat, maize, or Setaria), or the plant can be dicotyledonous (e.g., tomato, soybean, tobacco, potato, or Arabidopsis).

From Fig.1 and 1a, it can be seen that there is a gradual increase in the number of patents during 2010 to 2020. The number of patents reached maximum of ~2500 in 2019 and in 2020 it showing very progressive trend. Figure 2 the top patent assignees, including several leading academic institutions and corporations key word engineering plant genomes using CRISPR/Cas systems. The worldwide engineering plant genomes using CRISPR/Cas systems landscape depicted in figure 3. This research helps to recognize the R&D-intensive competitive landscape; to identify new innovations and developments in technology within an industry; to encourage better targeting of innovation and industrial policies; to determine their impact; and to identify networks of inventors and information flows within and between industries.

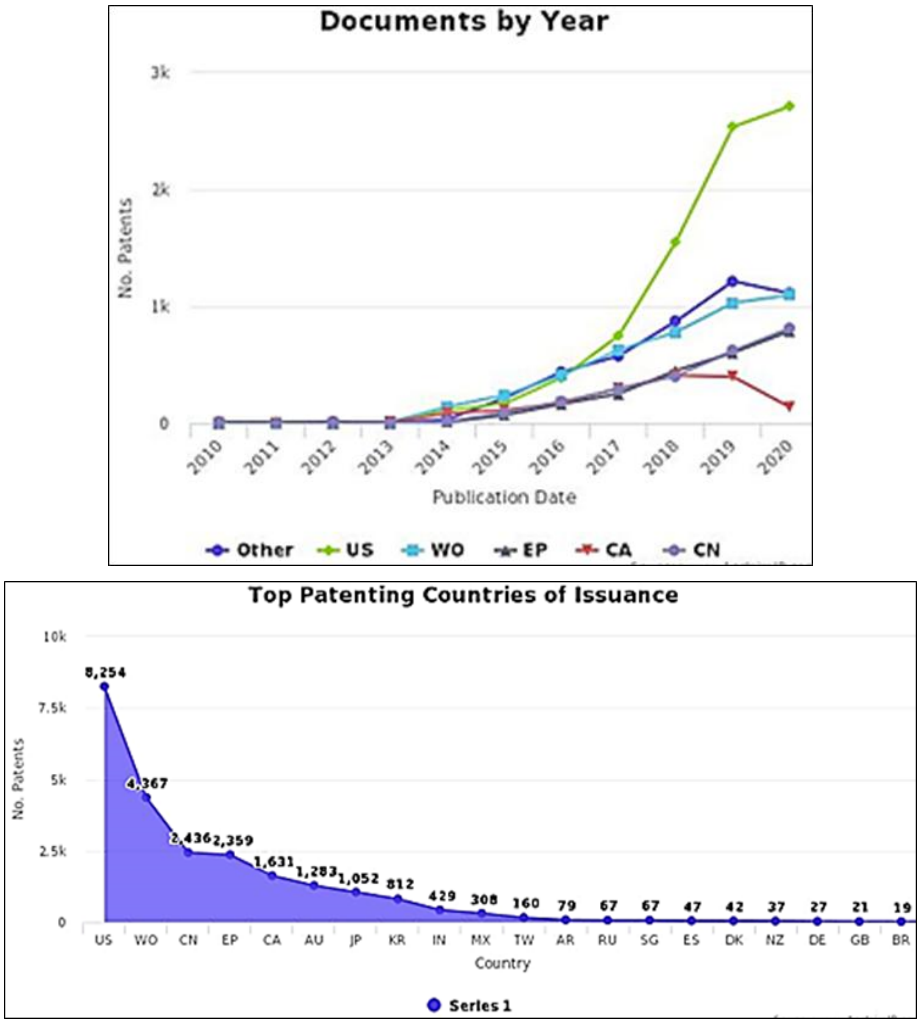


Fig 1: Depicts the number of CRISPR–Cas9 inventions, as represented by patent families, by year of original priority filing for each patent family, together with a count of subsequent foreign filings that expand already existing patent families. From 2013 there is notably increased activity. Geographical distribution of patent family filings by date of filing of the priority application for each invention in a given jurisdiction's patent office. Most inventions are filed in the United States is the leading country in the patenting issuance with a key word engineering plant genomes using CRISPR/Cas systems

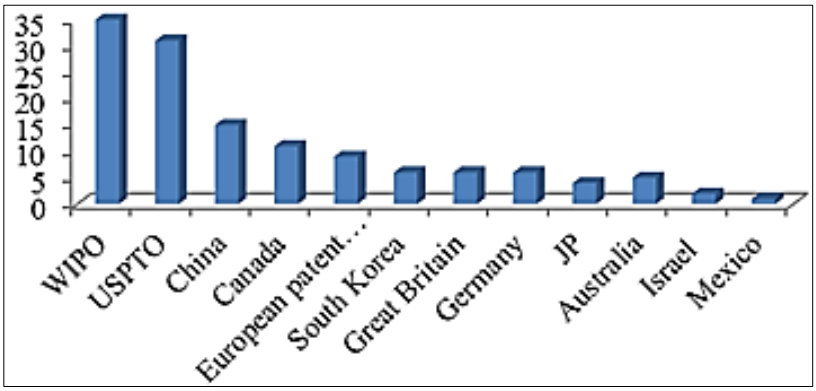
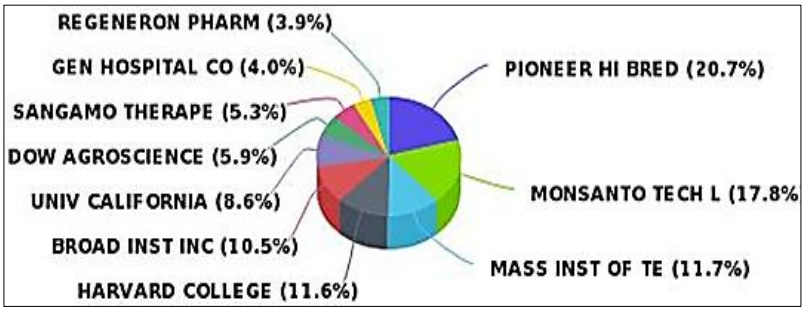


Fig 1a: Geographical distribution of patent family filings by date of filing of the priority application for each invention in a given jurisdiction's patent office



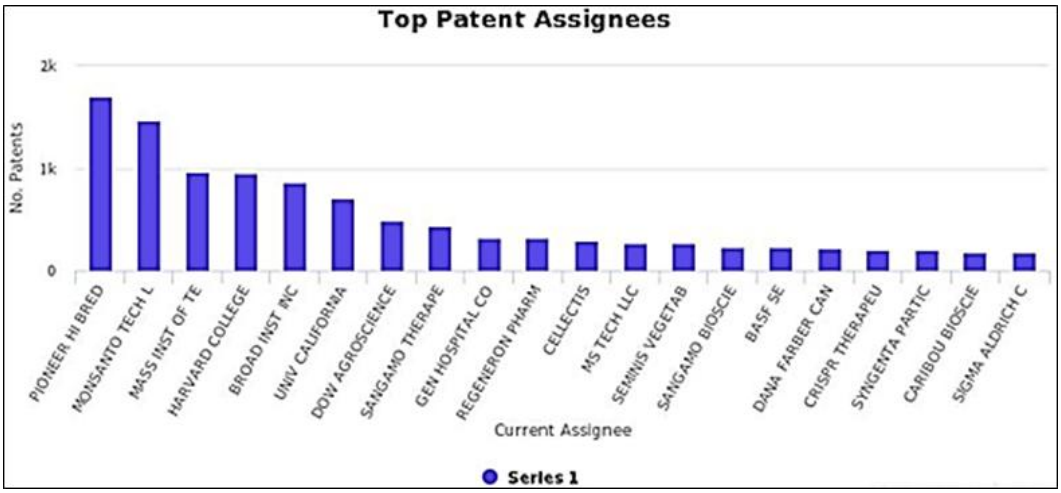


Fig 2: The top patent assignees, including several leading academic institutions and corporations key word engineering plant genomes using CRISPR/Cas systems

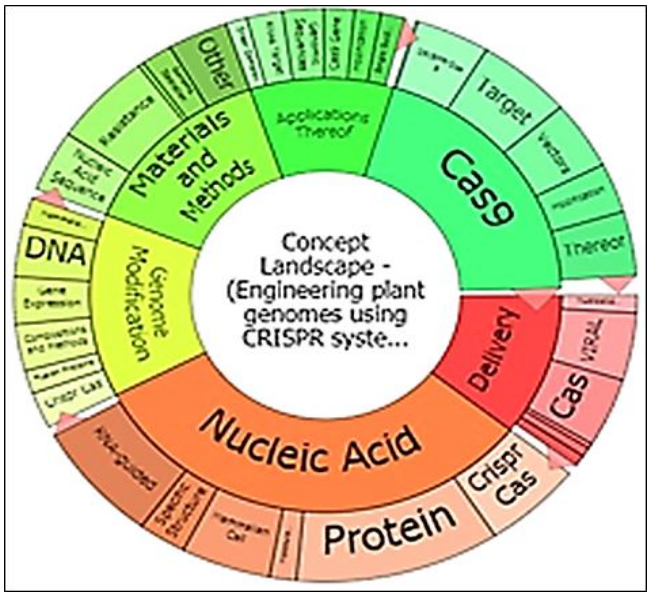


Fig 3: Depicts the worldwide engineering plant genomes using CRISPR/Cas systems landscape

Methods for non-transgenic genome editing in plants

Traditional plant breeding techniques have been established over several years to incorporate beneficial traits into plant species such as improved drought tolerance and crop yield. Such techniques have the downside that they usually need several successive rounds of crossing, and therefore it can take several years to effectively modify a particular plant phenotype. It became possible to engineer plants with genomic alterations with the advent of transgenic technologies by implementing transgene constructs and thus circumventing the need for standard plant breeding. Nevertheless these transgenic processes have also had many pitfalls. First, the insertion of transgenes into the genome (such as that mediated by Agrobacterium tumefaciens) is essentially spontaneous and may lead to numerous insertions that may create difficulties in tracking multiple transgenes present during segregation on various chromosomes. In addition, regardless of the chromosomal environment, transgene expression can be unstable, and transgene expression is silenced in certain cases. Moreover the cultivation of transgenic plants has proved to be a very contentious problem, often objected to the existence of such varieties by popular opinion, especially when the varieties in question are crops that will be planted in wide geographical areas and used as food for human consumption.

The first two of these issues can be solved by approaches that allow for selective alteration of the plant genome, making it possible to direct transgene insertions to single chromosomal sites conducive to gene expression, thus minimizing or removing the risk of multiple transgene insertions and silencing incidents. Targeted genome modification has been demonstrated in a number of species using engineered Zinc Finger Nucleases (ZFNs), which permit the creation of double stranded DNA break points at preselected loci and the subsequent insertion of transgenes in a targeted manner (Lloyd *et al.*, 2005; Wright *et al.*, 2005; Townsend *et al.*, 2009) [20, 35, 40]. A variant on this approach is to simply use the ZFN to establish a break point at a selected locus and then allow NHEJJ to repair the DNA (non-homologous end joining). Errors are also inserted into the freshly joined area throughout this step (e.g. nucleotide deletions) and this approach facilitates both the selective mutagenesis of chosen plant genes and the incorporation of transgene constructs. This keyword patent analysis addresses the field of plant molecular biology and in particular, offers non-transgenic methods for materials and methods for the processing of genome-engineered plants. Out of Fig. 4, it can be seen that the number of patents is growing steadily from 2010 to 2020. In 2018, the number of patents hit a high of 75K and there is a small decrease following terms.

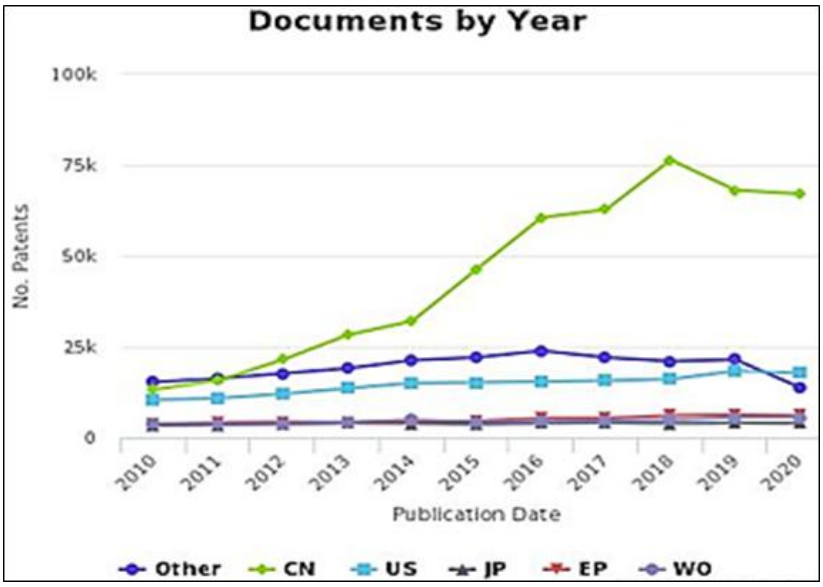


Fig 4: Depicts the number of CRISPR–Cas9 inventions, as represented by patent families, by year of original priority filing for each patent family, together with a count of subsequent foreign filings that expand already existing patent families. From 2019 there is notably increased activity

CRISPR vectors for plant transformation

In the discovery and production of highly critical agricultural traits, effective selective genomic editing holds a lot of promise. It was important to produce transforming lines that stably expressed the Cas9 and gRNA molecules in order to achieve modest efficiencies of targeted genome modification; in subsequent generations, the heritability of the resulting modifications was controlled. It is time-consuming to manufacture these stably expressing lines; thus to speed up and optimise the utility of this technology for trait discovery and growth, successful distribution methods are desperately needed. The lack of successful methods of distribution currently constitutes a significant obstacle to achieving highly efficient targeted alteration through plant species.

CRISPR Plant Cas9 vectors are designed for the transformation of Agrobacterium-mediated plants or for the bombardment of biolytic micro particles or the transformation of protoplasts. The products are based on the Streptococcus pyogenes-derived type IIA CRISPR-Cas9. In monocots and dicots, respectively, the native Cas9 coding sequence is codon optimised for expression. The monocot Cas9 constructs contain a monocot U6 promoter for sgRNA expression, and the dicot Cas9 constructs contain a dicot U6 promoter.

Principle

Bacteria and archaea use CRISPR/Cas structures as a protection against viruses and plasmids attacking them. The type II CRISPR/Cas system of the Streptococcus pyogenes bacterium has recently been engineered to operate in eukaryotic systems using two molecular components: a single Cas9 protein and a non-coding RNA guide (gRNA). A single gRNA can be used to programme the Cas9 endonuclease, directing a DNA double-strand break (DSB) at the desired genomic position. The cell then induces endogenous DNA repair processes, either non-homologous end joining (NHEJ) or homology-directed repair (HDR), analogous to DSBs caused by zinc finger nucleases (ZFNs), to cure the targeted DSB.

One-guide (sg) RNAs and Cas9 mRNA can be co-injected into a one-cell embryo in animals (Li *et al.*, 2013; Qin *et al.*, 2014) [28]. In plant duo, however it is difficult to perform this technique to the surrounding cell wall. Instead a process of gene transformation mediated by Agrobacterium was found to be sufficient for the delivery of a T-DNA containing sgRNA-Cas9 sequences into plant cells (Bortesi *et al.*, 2015) [3, 4]. One drawback of early CRISPR/Cas9 systems in plants is that only one or two target sites are typically identified by them. It has been shown, however that targeting several sgRNAs to the same gene will greatly improve the efficacy of editing (Bortesi *et al.*, 2010; Ma *et al.*, 2015) [3, 4]. By mixing multiple sgRNAs, the utility of Cas9 as a mutagen can also be increased by increasing the potency of removing large segments of the genome (Han *et al.*, 2014) [10]. It is often desirable to periodically locate genome editing at various locations simultaneously. To achieve this phenomenon in plants, several multi-site CRISPR/Cas9 systems have been developed (Zhang *et al.*, 2016; Ma *et al.*, 2015; Xing *et al.*, 2014; Lowder *et al.*, 2015; Wang *et al.*, 2015; Vad-Nielsen *et al.*, 2016; Vazquez-Vilar *et al.*, 2016) [21, 28, 36, 37, 38, 41, 43], For instance, the use of Golden Gate, Gibson Assembly, or compatible enzymes for multiplex gRNA cassette assembly. While these strategies have been shown, there are still a number of shortcomings in such techniques. Some methods can only assemble a few sgRNA expression cassettes (Xing *et al.*, 2014; Wang *et al.*, 2015) [28, 38], while others require several steps of subcloning (Lowder *et al.*, 2015; Vad-Nielsen *et al.*, 2016; Vazquez-Vilar *et al.*, 2016) [21, 36, 37]. The absence of standardisation to encourage open source acceptance and production of instruments is the common deficiency of these programmes (Hu *et al.*, 2019) [12]. Figure 5 reflects the number of CRISPR-Cas9 patents, as represented by patent families, with each patent family by year of initial priority filing, along with a number of corresponding international filings extending existing patent families. CRISPR/Cas vector systems for plant transformations are the top patent assignors, including many leading research organisations and companies. Global engineering CRISPR/Cas applications vectors for the landscape of plant transition (Fig. 6).

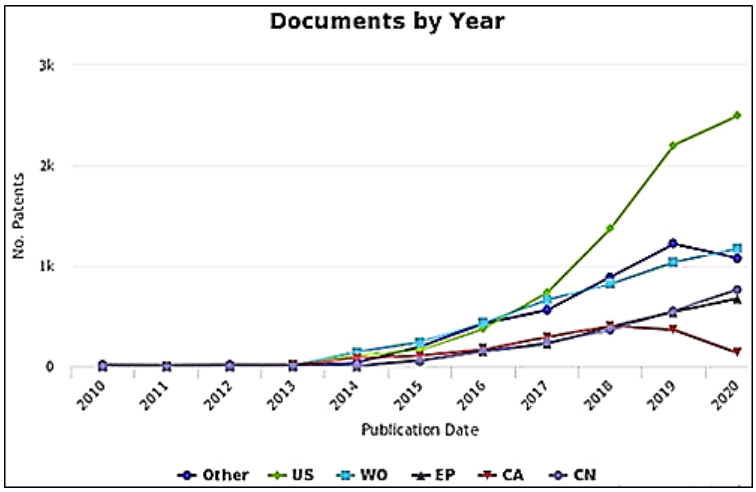


Fig 5: Depicts the number of CRISPR–Cas9 inventions, as represented by patent families, by year of original priority filing for each patent family, together with a count of subsequent foreign filings that expand already existing patent families

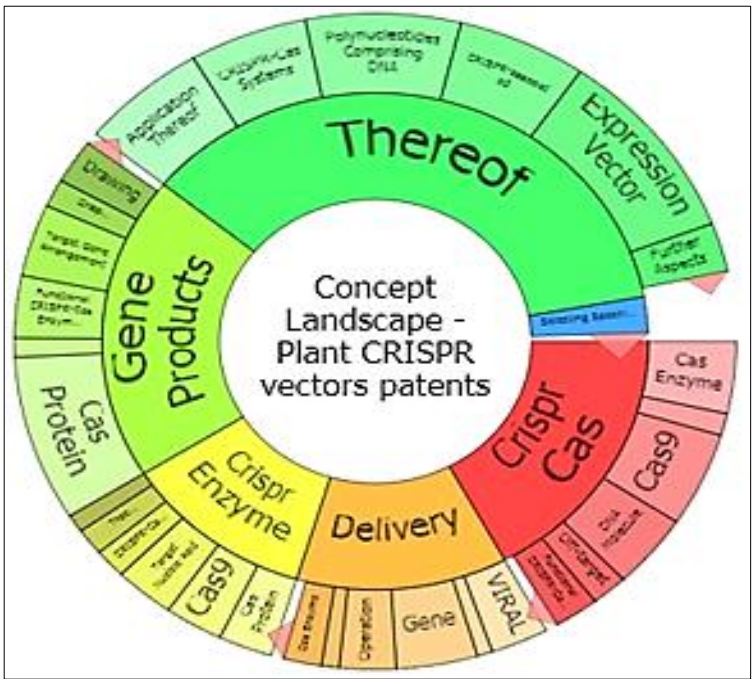


Fig 6: Depicts the worldwide engineering CRISPR/Cas systems vectors for plant transformation landscape

(1) DNA plasmid(s) encoding both Cas protein and sgRNA, (2) a Cas mRNA (which will be converted into Cas in planta) supplied with a separate sgRNA, and (3) RNP complexes consisting of the Cas protein and sgRNA, can be 'packaged' into various forms for delivery into plant cells (Ran *et al.*, 2017) [10]. Plant-specific RNA polymerase III promoters [AtU6 (*Arabidopsis*); TaU6 (wheat); OsU6 or OsU3 (rice)] are used to express Cas9 and gRNA in plant systems. There are several commercially available vectors for expressing Cas9 or Cas9 variants and gRNAs in plant systems.

Genome editing for virus resistance plants

Crop yield and production are seriously compromised by viral diseases, thus threatening global food security. For sustainable agriculture, genetic enhancement of plant virus resistance is important. To continue their life cycle, plant RNA viruses require host factors. CRISPR/Cas System-Mediated Plant Virus Resistance through Attacking Host Factors-Many genes that impart resistance to viruses are recessive (Kang *et al.*, 2005; Truniger and Aranda, 2009) [13], including eIF4E or eIF (iso) 4E4E4E initiation factors for eukaryotic translation (Lellis *et al.*, 2002; Nicaise *et al.*, 2003; Ruffel *et al.*, 2006) [17, 25, 30, 31]. The eIF4F-complex (eIF4E and eIF4G [or their isoforms] and eIF4A) and other host factors, such as the polyA-binding protein (PABP), bind to the potyviral 5' m7G cap structure and 3' polyA tail of mRNA for translation. The eIF4E and eIF (iso) 4E genes in the eIF4F complex connect with the 5' mRNA or viral RNA and each scaffold gene. Both the eIF4E and eIF (iso) 4E genes exist and have redundant roles in plant cytoplasm (Jackson *et al.*, 2010; Sanfacon, 2015; Wang and Krishnaswamy, 2012) [28, 38]. Viruses, especially potyviruses, can associate with one or both of those proteins, through the viral-encoded protein VPg (Duprat *et al.*, 2002; Hwang *et al.*, 2009; Ling *et al.*, 2009; Ruffel *et al.*, 2006; Sato *et al.*, 2005) [30, 31]. The copy numbers of the eIF4E and eIF (iso) 4E genes differ among plant species (Le Gall *et al.*, 2011). In Cucumis spp. (cucumber and melon), one gene each of eIF4E and eIF (iso) 4E have been identified (Rodriguez-Hernandez *et al.*, 2012; Gal-On *et al.*, unpublished) [29]. Both eIF4E and eIF (iso) 4E are recessive when mutated, and are essential for the translation of uncapped viruses having the VPg protein covalently linked to the viral RNA 5' (Wittmann *et al.*, 1997) [18, 39]. Genes eIF4E and eIF (iso) 4E interact with VPg in different hosts (Leonard *et al.*, 2000; Sanfacon, 2015; Jiang and Laliberte, 2011) and disruption of this link by mutagenesis or silencing prevents

virus infectivity (Duprat *et al.*, 2002; Lellis *et al.*, 2002; Rodriguez-Hernandez *et al.*, 2012; Sato *et al.*, 2005) [17, 29]. The interaction of normal mutations in the eIF4E and eIF (iso) 4E genes with potyvirus resistance has been observed in different crops and extended to breeding (Gomez *et al.*, 2009) [8]. Broad RNA virus resistance has been demonstrated by silencing of the eIF4E gene in tomato and melon (Mazier *et al.*, 2011; Rodriguez-Hernandez *et al.*, 2012) [25, 29]. The number of CRISPR-Cas9 discoveries, as represented by patent families, for each patent family by year of initial priority filing, along with a number of corresponding international filings that exceed existing patent families key word used for virus resistance plant genome editing (Fig. 7). Top patent assigned by Pioneer Hi Breed and Monsanto, plus several leading research institutions and companies for virus resistance plant key word genome editing (Fig. 8).

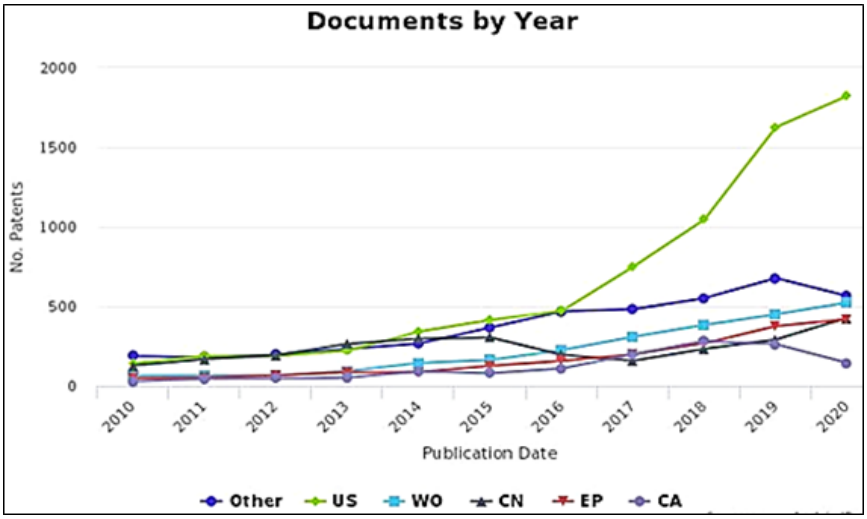


Fig 7: Depicts the number of CRISPR–Cas9 inventions, as represented by patent families, by year of original priority filing for each patent family, together with a count of subsequent foreign filings that expand already existing patent families key word used genome editing for virus resistance plant

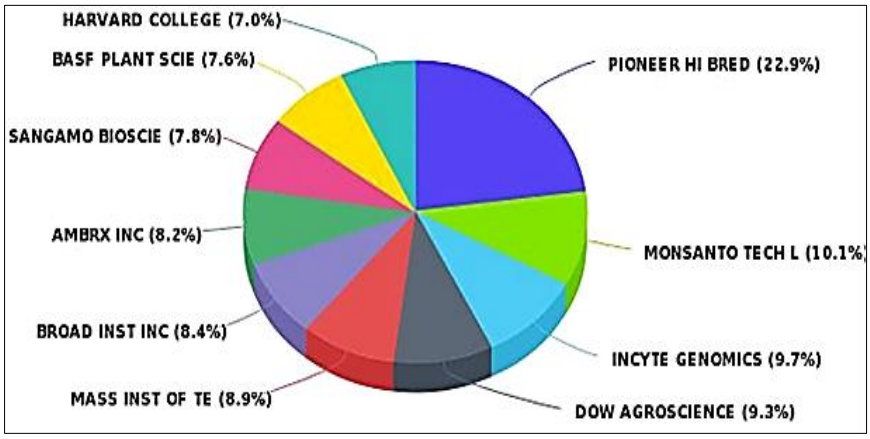


Fig 8: The top patent assignees, including several leading academic institutions and corporations key word genome editing for virus resistance plant

CRISPR plant sgRNA

Most recently a new gene targeting technique has been developed in microbial and mammalian systems based on the nuclease mechanism associated with the cluster of frequently interspaced short palindromic repeats (CRISPR). The CRISPR-associated nuclease is part of adaptive immunity in bacteria and archaea. The Cas9 endonuclease, a component of Streptococcus pyogenes type II CRISPR/Cas system, forms a complex with two short RNA molecules called CRISPR RNA (crRNA) and transactivating crRNA (transcrRNA), which guide the nuclease to cleave non-self DNA on both strands at a specific site. The crRNA-transcrRNA heteroduplex could be replaced by one chimeric RNA (so-called guide RNA (gRNA)), which can then be programmed to targeted specific sites. The minimal constrains to program gRNA-Cas9 is at least 15-base-pairing between engineered 5′-RNA and targeted DNA without mismatch, and an NGG motif (so-called protospacer adjacent motif or PAM) follows the base-pairing region in the targeted DNA sequence. Generally, 15-22 nt in the 5′-end of the gRNA region is used to direct Cas9 nuclease to generate DSBs at the specific site. The CRISPR/Cas system has been demonstrated for genome editing in human, mice, zebrafish, yeast and bacteria. Distinct from animal, yeast, or bacterial cells to which recombinant molecules (DNA, RNA or protein) could be directly transformed for Cas9-mediated genome editing, recombinant plasmid DNA is typically delivered into plant cells via the Agrobacterium-mediate transformation, biolistic bombardment, or protoplast transformation due to the presence of cell wall. Thus, specialized molecular tools and methods need to be created to facilitate the construction and delivery of plasmid DNAs as well as efficient expression of Cas9 and gRNAs for genome editing in plants. Furthermore, Cas9-gRNA recognizes target sequence based on the gRNA and DNA base pairing that may have a risk of off-targeting. Therefore it is also critical to determine the parameter for designing Cas9-gRNA constructs with minimal off-target risk for plant genome editing. Due to these significant differences

between animals and plants, it is still unknown if the CRISPR-Cas system is functional in the plant system and if it can be exploited for specific gene targeting and genome editing in crop species. sgRNA is an abbreviation for "single guide RNA." As the name implies, a sgRNA is a single RNA molecule that contains both the custom-designed short crRNA sequence fused to the scaffold tracrRNA sequence. sgRNA can be synthetically generated or made *in vitro* or *in vivo* from a DNA template. the number of CRISPR–Cas9 inventions, as represented by patent families, by year of original priority filing for each patent family, together with a count of subsequent foreign filings that expand already existing patent families with keyword plant sgRNAs (Fig. 9). The top patent assignees, including several leading academic institutions and corporations key word plant sgRNAs is depicted in figure 10. Worldwide engineering CRISPR/Cas plant sgRNAs landscape is presented in figure 11.

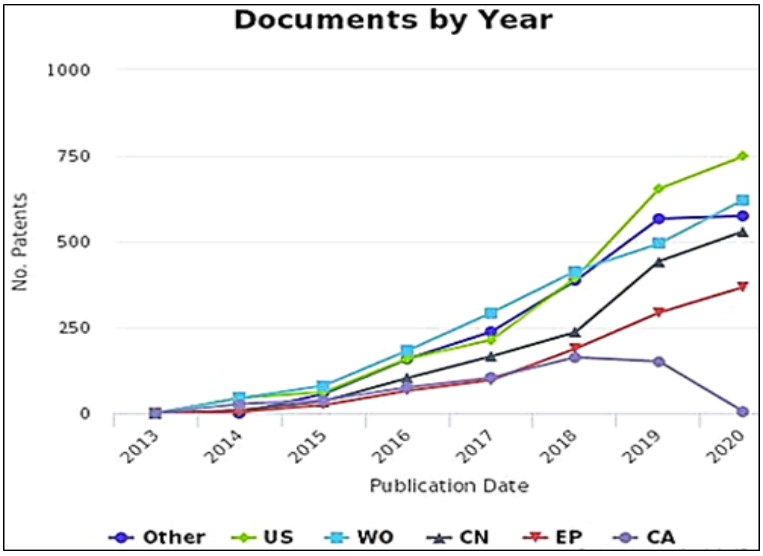


Fig 9: Depicts the number of CRISPR–Cas9 inventions, as represented by patent families, by year of original priority filing for each patent family, together with a count of subsequent foreign filings that expand already existing patent families with keyword plant sgRNAs

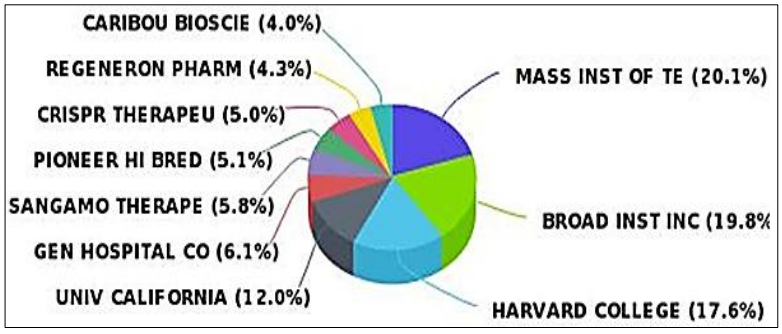


Fig 10: The top patent assignees, including several leading academic institutions and corporations key word plant sgRNAs

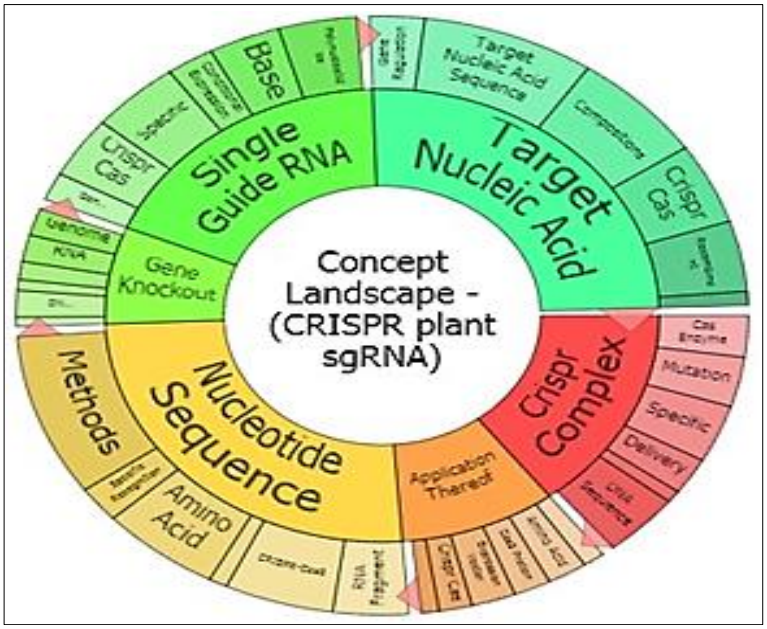


Fig 11: Depicts the worldwide engineering CRISPR/Cas plant sgRNAs landscape

Indian context of CRISPR/Cas9 based genome editing in crops

In recent years, Indian patent activity has seen tremendous progress, in particular in the filing of patents and patents issued under the U.S. patent system. In various technical areas/sectors, this development has been observed. It remains to be discussed to what degree India's patenting operation has produced an influence within the technical world. While attempts have been made to use the CRISPR technology for crop improvements, there has not been a patent issued and accepted until now when it comes to patents. CRISPR order for crop enhancement at the infancy stage. The CRISPR patenting practice should be given a lot of emphasis.

Current and future development

From cereals to vegetables that reduce crop yields and pose a significant threat to food security, plant viruses infect many essential crops in agriculture, feeding the increasing world population. The pressure of the new global threat to food security lies in the need to increase crop production. In this respect, biotechnology stands out as an important method for producing plants capable of dealing with pests, diseases and harsh climatic environments and utilizing natural resources more effectively. Targeted alteration of plant genomes is a powerful technique for research and engineering in cellular systems, paving the way for significant, novel agricultural traits to be identified and created. For the study of plant gene regulation, the capacity to produce genetic mutations is vital. In recent years, attempts have been made to adapt the CRISPR/Cas9 method for its use in crop plants.

Agricultural scientists have been developing plants by biotechnology for over 25 years by moving genes from one plant species (or bacteria) to another. The tight regulation and years of testing necessary are needed for so-called genetically modified organisms (GMOs). Yet without needing to pass new genes from one cell to another, newer gene-editing methods such as CRISPR produce the same results. Due to its simplicity, quality and flexibility, this has received much interest. This technique is used in almost all fields of biological science and has a broad variety of applications in biomedicine, healthcare, and agriculture. In many agricultural plant species, researchers are using CRISPR/Cas9 by targeting different genes of interest for better feeding, enhanced susceptibility to diseases and improved drought tolerance.

Genome editing is an important method in fundamental biological science and, considering its promise for a wide variety of future commercial uses, has been sought for years. Academic institutions' patent filings citing key components of the CRISPR-Cas technology have raised fear among scientists and legal experts that they could prevent or slow down the technology's production and usage by maintaining exclusive control over what can be called an important research tool (Egelie *et al.*, 2016). While important achievements in the application of CRISPR were made in crop improvements, patents and patentable inventions remained very limited to those geographical regions. Patents and patentable inventions must be stressed and this will allow CRISPR technology for food, nutrition and health protection. Patent documentation relating to the use of CRISPR in seed enhancement is a rich source of commercially available scientific information and thus, patent analytics is considered a valuable vehicle for R&D management. Analyzing these patents according to different requirements will offer useful knowledge that can be used in various ways. These indices may be used to evaluate up-to-date technological patterns and find promising areas for the production of new goods. Patent quest and study of the formulation of technological strategies may contribute to a greater likelihood of success in new technological projects. Finally, the basic purpose of CRISPR for patent study of crop improvement is to find, produce, and understand new products and methods for an effective and sustainable system of crop production. It has also been noted that patenting practices are rapidly developing in new technology fields. This speeds up the process of technology assessment for organizations and helps to demonstrate with better certainty the major technical paths and holes for further R&D decisions. Opportunities are enormous and the sky is the limit for researchers interested in taking advantage of R&D in CRISPR technology to develop agricultural crops.

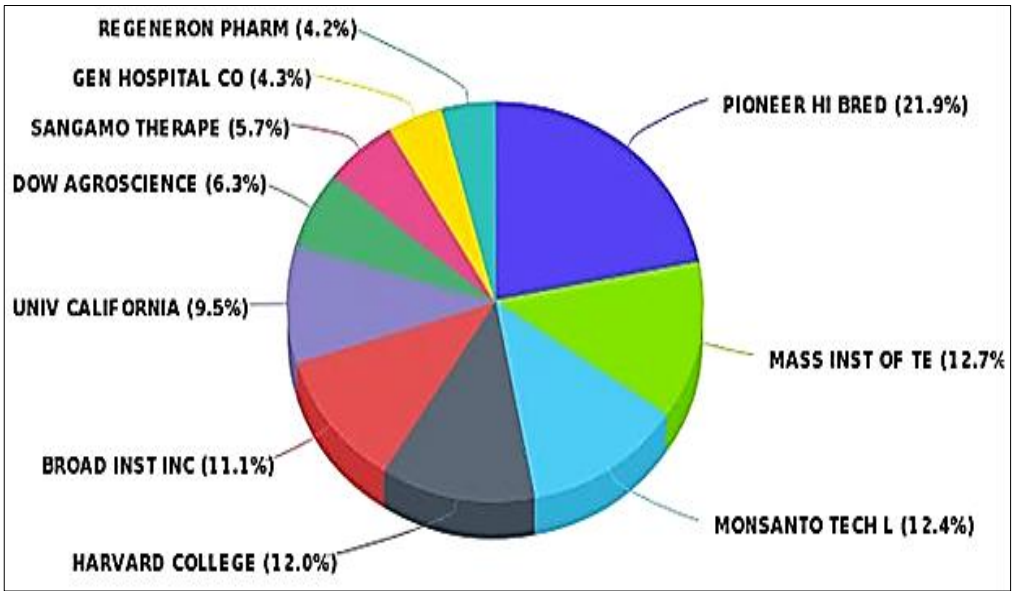
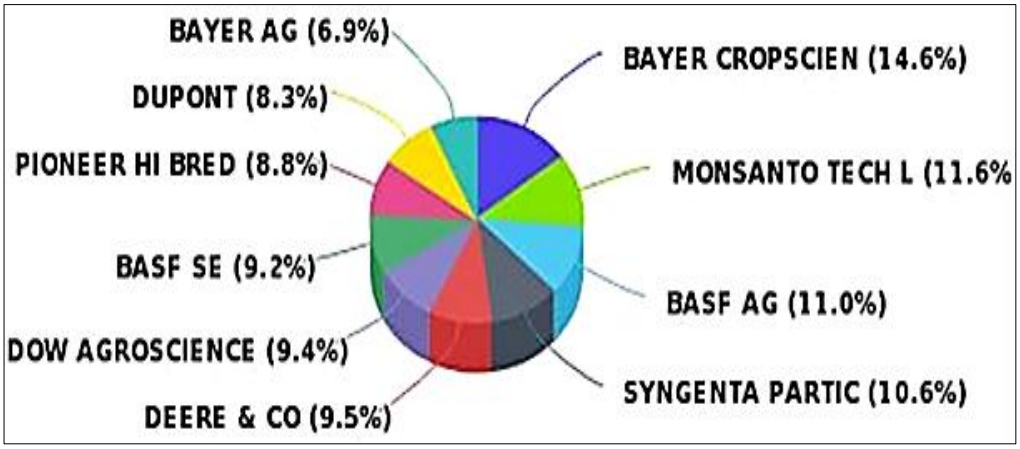


Fig 12: The top patent assignees, including several leading academic institutions and corporations key word CRISPR/Cas vector systems for plant transformations



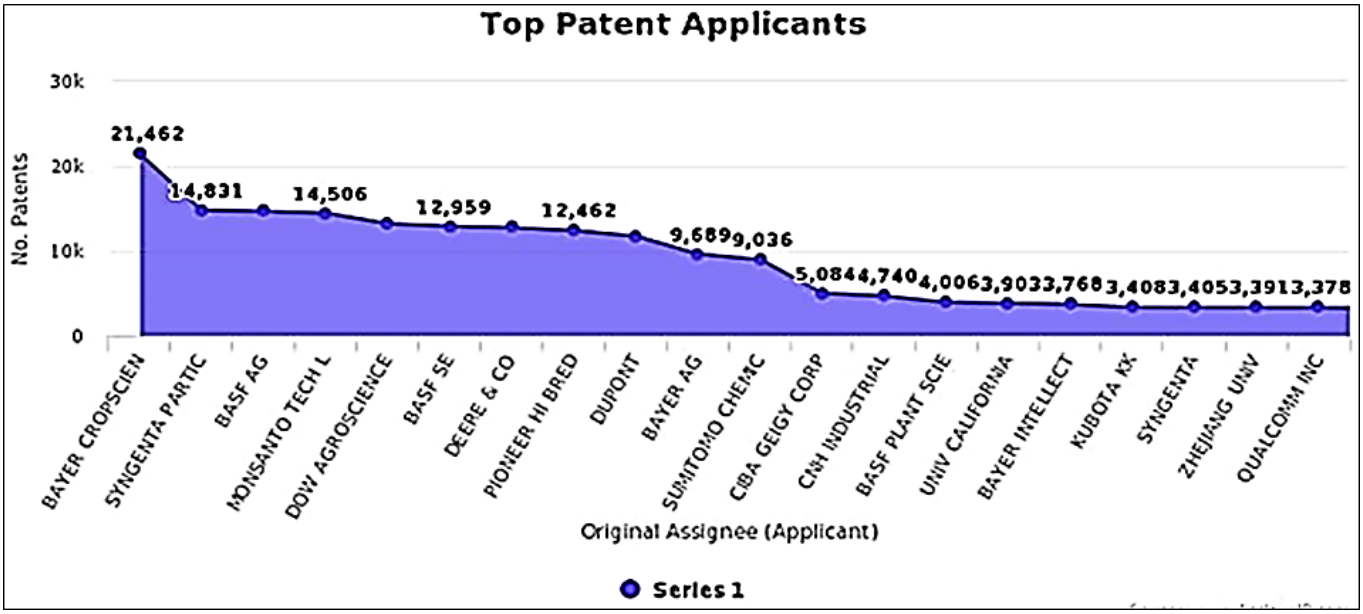


Fig 13: The top patent assignees, including several leading academic institutions and corporations key word engineering plant genomes using CRISPR/Cas systems

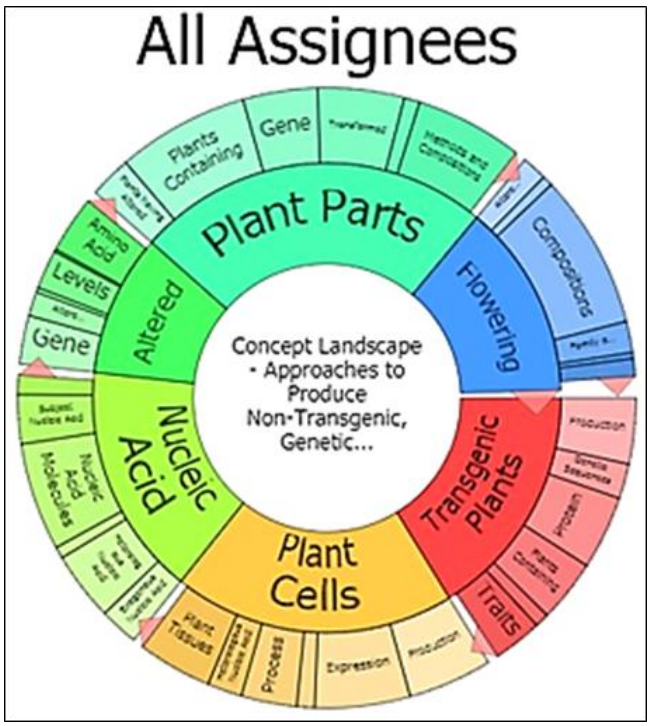


Fig 14: Depicts the worldwide engineering plant genomes using CRISPR/Cas systems landscape with keyword approaches to produce non-transgenic genetic plants

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