CiteSpace-Based Review of Agricultural Biotechnology Applications

Caixia Zhang [1, a]

[1]School of Business, University of Jinan, Jinan, ShanDong 250002, China

[a] se_zhangcx@ujn.edu.cn

Abstract. Agricultural biotechnology holds the potential to enhance crop productivity, improve food quality, and promote sustainable agricultural practices. With Web of Science (WoS) core collection as the data source, this study applies CiteSpace to visualize research frontiers, hot topics, and knowledge flow in agricultural biotechnology applications. The findings reveal an increasing number of publications and frequent regional collaborations between China, USA and India in this field from 2011 to 2023. It integrates diverse disciplines such as plant sciences, biotechnology, applied microbiology, genetics, and microbiology, encompassing a broad range of topics. CRISPR, CRISPR/Cas9, and strawberry emerge as the main knowledge sources in this domain. Additionally, the application of genetically modified crops using technologies like the CRISPR system, base editing, and prime editing may emerge as a novel research trend in the future, providing valuable insights and ideas for further exploration in the field of agricultural biotechnology applications.

Keywords: agricultural biotechnology; bibliometric; genetically modified; CRISPR/Cas9.

1. Introduction

Agricultural biotechnology applies scientific techniques such as biology, genetics, and molecular biology to modify and regulate the genetic information of plants, animals, and microorganisms in order to enhance crop yield, quality, and resistance. Its significance can be seen in maintaining global food security and achieving sustainable agricultural development [1]. Bt (Bacillus thuringiensis) has caused a 24% increase in cotton yield per acre through reduced pest damage and a 50% gain in cotton profit among smallholders in India [2]. Additionally, developing countries have experienced higher growth in crop yield and farmer profits compared to developed countries [3]. According to ISAAA 2019 database, a total of 525 transgenic events in 32 crops have been commercialized.

The research outcomes of agricultural biotechnology applications are varied, and the research topics are wide-ranging. Therefore, conducting a systematic summary of past research achievements, identifying the forefront and hot topics, and establishing a knowledge flow for the application of agricultural biotechnology can help researchers gain a more comprehensive understanding of the field and find new directions for future research.

The structure of this study is as follows: section 2 describes the data sources and research methods, followed by section 3 which applies the knowledge map method to examine the collaboration of authors, institutions and countries. Co-citation cluster analysis is employed to identify research frontiers and hotspots, and to present knowledge flow. Section 4 provides a summary of the work and its limitations.

2. Data Sources and Methods

This study primarily examines the utilization of gene editing technology in crops, investigating its applications based on three distinct sets of search keywords outlined in Table 1. These sets of keywords are combined using the logical operator "AND" to refine the search utilizing data retrieved from the Web of Science core database covering the timeframe from 2011 to 2023. The search is limited to literature types such as articles or reviews written in English, while excluding materials, book chapters, letters, and editorials. Consequently, a comprehensive dataset consisting of 9560 records was obtained for further analysis.

Table 1. Keywords for Web of Science (WoS) Query

Group	Topic	Keywords
1	biotechnology	Genome editing technology; Genetically modified; Genetic modification; Genome engineering; genome editing; Clustered Regularly Interspersed Short Palindromic Repeats; CRISPR system; CRISPR-Cas9; TALEN; ZFN; sgRNA
2	Trait enhancement	Trait modification; Trait improvement; Trait engineering; Disease resistance; Pathogen resistance; Pest resistance; Immunity enhancement; Yield improvement; Higher crop yield; Nutritional enhancement; Biofortification;Environmental stress tolerance; Abiotic stress resistance; Climate resilience
3	Agricultural crops	Agricultural varieties; Agronomic crops; Cultivated plants; Crops in the field; Field crops; Plants grown in farmland

Knowledge map is the main method of bibliometric visualization, which can comprehensively analyze the research status in various fields, examine development trends and frontier areas. The software CiteSpace (6.2.R4 Advanced) is used as a bibliometric and visual analysis tool in the field of agricultural biotechnology applications, which can perform co-citation analysis, burst detection and cluster analysis in this paper.

3. Results and Analysis

3.1 Researchers Analysis

Co-occurrence analysis involves the computation of co-occurrence frequencies and degree centrality based on data gathered from multiple literature sources. By constructing co-occurrence matrices and network graphs, the co-occurrence relationships among diverse authors, institutions, or countries can be established. This analytical approach facilitates researchers in developing a comprehensive comprehension of collaboration and collaboration patterns within the realm of agricultural biotechnology research. Additionally, it aids in identifying potential collaborative partners and uncovering the dynamics inherent in the research field.

Table 2. Leading authors, institutions, and countries based on frequency and centrality.

Cited Authors	Frequency	centrality	Institutions	Frequency	centrality	Country	Frequency	centrality
JINEK M	1144	0.07	Chinese Academy of Agricultural Sciences	568	0.03	China	2923	0.02
ZHANG Y	925	0.11	Chinese Academy of Sciences	461	0.07	USA	2294	0.13
SHAN QW	914	0.11	Ministry of Agriculture & Rural Affairs	336	0.04	India	752	0.08
CONG L	767	0.02	University of California System	258	0.02	Germany	707	0.07
MA XL	733	0.00	Huazhong Agricultural University	254	0.06	Japan	484	0.02
XIE KB	684	0.03	Indian Council of Agricultural	241	0.10	England	448	0.06

			Research (ICAR)					
NEKRASOV V	683	0.06	INRAE	227	0.03	South Korea	403	0.05
WANG YP	665	0.04	China Agricultural University	211	0.03	Australia	395	0.11
LIANG Z	596	0.08	UDICE-French Research Universities	210	0.01	France	375	0.09
FENG ZY	592	0.00	University of Chinese Academy of Sciences	210	0.02	Brazil	364	0.05

Table 2 shows that JINEK M has the highest frequency value among authors, with a cited frequency of 1144. Similarly, ZHANG Y and SHAN QW possess the highest centrality value among authors, with a centrality of 0.11. Moving on to institutions, the Chinese Academy of Agricultural Sciences holds the top frequency value, standing at 568. Meanwhile, the Indian Council of Agricultural Research secures the highest centrality value among institutions, with a centrality of 0.10. Finally, in terms of countries, China ranks first with the highest frequency value of 2923, while the USA claims the highest centrality value among countries, boasting a centrality of 0.13. These authors, institutions, and countries with the highest frequency and centrality values represent the most active, influential, and important roles within the research network. Their research output is frequently cited and they hold a high position and influence within the research network. These results can be used to evaluate their academic contributions and leadership positions in agricultural biotechnology research.

3.2 Keywords Analysis

Keyword clustering and bursts detecting complement each other in helping researchers gain a comprehensive understanding of the evolution and dynamics of the agricultural biotechnology research topics and hotspots, providing support for further research and decision-making.

Fig. 1 Keyword clustering timeline of agricultural biotechnology research

Figure 1 illustrates the largest eight clusters formed by clustering the keywords of agricultural biotechnology based on subject. Agricultural biotechnology, as an interdisciplinary field, integrates the fundamental theories and knowledge base of plant sciences, biotechnology and applied microbiology, genetics, and microbiology. Simultaneously, agricultural biotechnology also applies

to practical domains such as environmental sciences, entomology, biodiversity conservation, and food science and technology, in order to promote sustainable development in agricultural production and food processing. Advances in agricultural biotechnology will stimulate further research in the fundamental scientific disciplines, while applied scientific disciplines may develop new methods and technologies to support the application of agricultural biotechnology.

The keywords: genetically modified crops, gene flow, bacillus thuringiensis, genetically modified organisms, zinc finger nucleases, quantification, corn, risk assessment, maize, and crops, all experienced citation bursts during the year 2011. These burst periods continued until 2018, indicating a sustained and ongoing interest in these respective topics. Among them, "genetically modified organisms" and "risk assessment" completed their burst periods in 2016, while "bacillus thuringiensis (Bt)" and "zinc finger nucleases (ZFNs)" concluded their bursts in 2016 and 2017, respectively. Notably, "genetically modified crops" exhibited the highest burst strength with a value of 51.72, indicating an exceptional level of research attention compared to the average citation rate.

The research hotspots in agricultural biotechnology include the utilization of Bt, ZFNs, and their applications in creating genetically modified crops or gene therapy for genetic diseases such as corn. Due to the transmission and exchange of genetic material among different individuals or populations, gene flow may occur in genetically modified crops, raising questions about the traceability of transgenic genotypes. Therefore, it is necessary to use quantitative methods to measure and assess the presence and level of transgenic material, and analyze and evaluate aspects such as environmental risks, food safety, and bio-safety associated with the introduction of genetically modified crops or other gene editing techniques.

3.3 Knowlege Flow and Prospect

By utilizing co-occurrence analysis of cited references and clustering based on literature titles, a network can be formed to reveal connections and associations between research articles to gain insights into the forefront topics and important concepts in agricultural biotechnology applications. Additionally, cluster dependencies, which refer to potential interdependencies between different clusters obtained through clustering algorithms, enable us to understand the knowledge flow in agricultural biotechnology, and provide a more comprehensive perspective for further research.

In Fig. 2, the Co-citation cluster dependencies of agricultural biotechnology research are illustrated. The arrows represent knowledge sources, while the tails represent cited references. The cluster formed by co-cited references includes CRISPR, CRISPR/Cas9, susceptibility genes, strawberry, base editing, Cas12a, abiotic stress, soybean, and homologous recombination. Among these, the arrows pointing to CRISPR, CRISPR/cas9, and strawberry represent the foundational research in agricultural biotechnology.
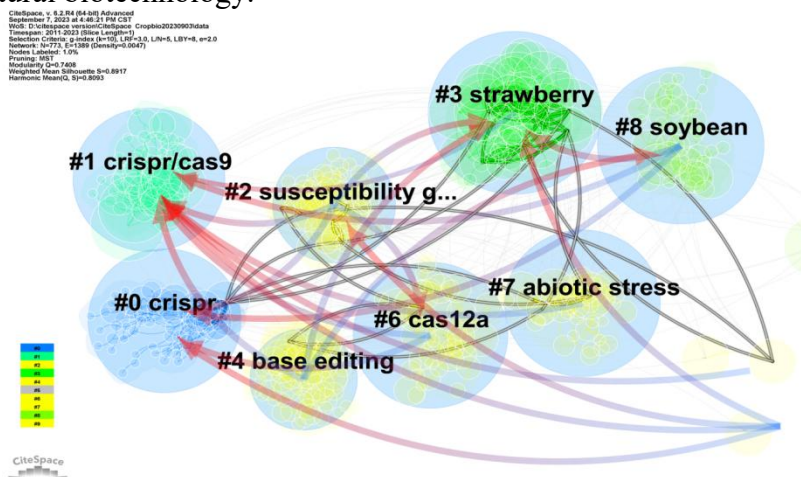

Fig. 2 Co-citation cluster dependencies of agricultural biotechnology research

3.3.1 Forefront Topics

Genome editing has been developed to obtain desired plant traits, as it could generate precise genome modification. Many systems have been developed to achieve genome editing in plants, including ZFNs, transcription activator-like effector nucleases (TALENs) and the CRISPR/Cas system [4]. CRISPR is a family of DNA sequences found in the genomes of prokaryotic organisms, functioning as part of their immune system to protect against viral infections. The CRISPR family includes new members like CRISPR/Cas9, Cas12a(Cpf1), Cas12b and Cas13(C2c2), which make gene editing more efficient, precise, and accessible. It holds great potential in agriculture by improving crop traits for increased productivity and disease resistance. CRISPR/Cas system has become one of the most widely used systems due to its low-cost, easy-to adapt and high specificity during genetic manipulation. Since 2013, the CRISPR/Cas genome editing system has been extensively utilized in various plants, resulting in remarkable advancements in the genetic enhancement of several agronomically significant fruit crops. This includes climacteric fruits such as apples, bananas, tomatoes, kiwifruits, and peaches. Additionally, non-climacteric fruits like strawberries, grapes, watermelons, cucumbers, and citrus fruits have also experienced significant progress in genetic improvement through the application of CRISPR/Cas technology.

Using the CRISPR/Cas9 system, several gene editing studies have been conducted in strawberries to explore fruit development mechanisms and enhance fruit quality. Some research has focused on the regulation of fruit growth by auxin and gibberellins, where the knockout or mutation of related genes was found to affect the morphology and ripening process of strawberries. In addition, gene editing has also been employed to regulate sugar content and color in the fruit, aiming to improve the taste and appearance of strawberries. These studies provide new possibilities and candidate gene resources for genetically edited strawberry breeding.

CRISPR/Cas9 has been applied to enhance the resistance of plants to abiotic stress, such as iron deficiency, cold stress, and herbicide resistance. Studies have shown that editing related genes through the CRISPR/Cas9 system can increase the expression of defense genes in plants and improve their response to stress. For example, mutation of the cyclic nucleotide-gated ion channel gene MdCNGC2 in apples increased the expression of defense-related genes. Additionally, knockdown of the SQUAMOSA promoter-binding protein-like gene SlSPL-CNR in tomatoes enhanced the plant's response to iron deficiency. Moreover, editing cold-responsive genes such as SlCBF1 and VaPAT1 in plants can also affect their response to cold stress. Furthermore, editing the EPSPS and ALS genes in plants can enhance their resistance to herbicides [5]. These studies provide new approaches to using gene editing techniques to improve the stress tolerance of plants.

3.3.2 Prospects and Challenges

Future agricultural biotechnology research will focus on the development of new technologies that activate gene expression by introducing exogenous transgenic components. For example, base editors will be used to generate programmable single nucleotide changes in DNA, while prime editors will enable the insertion of long DNA fragments without transgenic over-expression. These advancements aim to provide powerful and precise tools for genetic manipulation, harvest plants with a combination of beneficial traits and the elimination of unfavorable traits, and accelerate crop breeding.

CRISPR/Cas has made significant progress in generating desired edits at target sites. The future directions of CRISPR technology involve further refining the accuracy and efficiency of gene editing, reducing off-target effects, and extending its applicability to more complex organisms [6]. Meanwhile, the CRISPR/Cas system is a powerful programmable platform that offers flexibility and scalability in simultaneously regulating multiple genes or precisely modifying genes at the epigenetic level. fusion of the catalytically inactive Cas variants (dCas) with various enzymes , including CRISPR-mediated gene activation (CRISPRa), interference (CRISPRi), and epigenetic modifications，has expanded the scope of CRISPR/dCas systems. Engineered dCas9, dCas12a, and dCas12b fused with different enzymes can serve as effective gene regulation platforms.

CRISPR will be expanded as a tool for crop breeding to ensure future food security, in response to the complex global impacts of the COVID-19 pandemic, economic uncertainty, and climate change. Its application in improving crop breeding and addressing food security issues is particularly evident in the breeding of rice, wheat, and soybean, where it accelerates the lengthy process of generating male-sterile lines in hybrid breeding and enhances the potential of crops to adapt to marginal soils, which frequently occur in developing or impoverished countries, are characterized by their poor fertility and environmental constraints on crop growth and yield.

However, the barriers to the wide application of agri-biotechnologies are multifaceted, encompassing not only technical challenges such as the development of complex tissue culture protocols [7], low transformation efficiency, species-dependence and transgene integration into the host genome, but also economic bottlenecks. The scattered Intellectual property ownership related to various aspects of CRISPR-Cas technology, including gRNA guide sequences, nucleases, vector delivery, and CRISPR sequences, has created a complex patent licensing and commercialization process that poses a significant barrier to its implementation in plant science [8]. This complexity limits the ability of biotechnology companies, particularly smaller ones, to develop and apply CRISPR technology in innovative research and development. As a result, the commercialization and advancement of CRISPR technology in plant science are hindered [9]. Policymakers and industry stakeholders must work together to address this issue and create a more favorable environment for innovation and development in the field.

4. Conclusions and Discussion

Agricultural biotechnology plays an essential role in increasing crop yield, addressing food security challenges, and reducing pesticide use and environmental impact. This study utilizes CiteSpace to conduct visual analysis on literature related to the application of agricultural biotechnology from 2011 to 2023 from the perspectives of collaboration networks, co-citation analysis, and co-occurrence analysis. It finds (1) JINEK M, ZHANG Y and SHAN QW are the leading researchers, CAAS and ICAR are pioneer institutions in this field, and China, USA and India play important roles within the research network. (2) CRISPR, CRISPR/Cas9, and strawberry emerge as the main knowledge sources in this domain. (3) Through keyword co-occurrence analysis, it finds that genetically modified crops, gene flow, BT, risk assessment, maize, and corn is a research hotspot. In recent years, CRISPR system, base editing, and prime editing emerge as the most explosive keyword and will become a major focus of future research.

This study contributes to valuable insights and ideas for further exploration in agricultural biotechnology applications. However, it still has certain limitations. It did not conduct a full-text qualitative analysis but solely relied on quantitative analysis based on bibliometric tool - CiteSpace. Therefore, there may be some information gaps. Additionally, the large number of literature sources results in information overload, requiring further interpretation and explanation.

Acknowledgement

References

[1] Kovak, E., Blaustein-Rejto, D., & Qaim, M. (2022). Genetically modified crops support climate change mitigation. Trends in Plant Science, 27(7), 627-629.

[2] Kathage, J., Qaim, M. Economic impacts and impact dynamics of Bt (Bacillus thuringiensis) cotton in India. Proceedings of the National Academy of Sciences, 2012, 109(29): 11652-11656.

[3] Areal, F. J., RIESGO, L., Rodriguez-Cerezo, E. Economic and agronomic impact of commercialized GM crops: a meta-analysis. The Journal of Agricultural Science, 2013, 151(1): 7-33.

[4] Zhou, J., Wang, G., & Liu, Z. Efficient genome editing of wild strawberry genes, vector development and validation. Plant biotechnology journal, 2018, 16(11): 1868-1877.

[5] Ma, Z., Ma, L., & Zhou, J. Applications of CRISPR/Cas genome editing in economically important fruit crops: recent advances and future directions. Molecular Horticulture, 2023, 3(1): 1-29.

[6] Van Beljouw, S. P., Sanders, J., Rodríguez-Molina, A., et al. RNA-targeting CRISPR–Cas systems. Nature Reviews Microbiology, 2023, 21(1): 21-34.

[7] Kleter, G. A., van der Voet, H., Engel, J., et al. Comparative safety assessment of genetically modified crops: focus on equivalence with reference varieties could contribute to more efficient and effective field trials. Transgenic Research, 2023: 1-16.

[8] Miglani, G. S. Genome editing in crop improvement: Present scenario and future prospects. Journal of Crop Improvement, 2017, 31(4): 453-559.

[9] Goodman, D. Agri-Biotechnology and the Failed Promises of the Seed-Chemical Complex, CRISPR and Gene Editing, and Regulatory Capture. In Transforming Agriculture and Food ways. Bristol University Press, 2023: 53-70.