# VII. Agriculture

# 1 Engineering research fronts

## 1.1 Trends in Top 10 engineering research fronts

The Top 10 engineering research fronts in the field of agriculture mainly include: ① research on biological mechanisms and mechanisms related to crop breeding, such as "crop pan-genome" and "functional gene identification by multi-omics in animals"; 2 research on improving the quality, yield, and green production of animal and plant products, such as "mechanisms and methods for synergistic improvement of crop yield, quality, and efficiency", "genetic basis and regulatory network for the quality formation of horticultural crops", "intelligent identification mechanism and real-time monitoring technology for crop diseases and pests", "diagnosis of forest diseases and pests based on deep learning", and "straw modification and rapid decomposition technology"; ③ animal medicine and nutrition are still at the forefront of research in animal husbandry, such as "mechanisms of host inflammatory response regulation mediated by significant animal pathogens", as well as "antibiotic-free nutritional regulation techniques for the intestinal health and growth of livestock and poultry". The number of core papers in the engineering research fronts in agriculture ranges from 18 to 67, with an average of 47 papers, similar to previous years. The citations per paper range of articles is 28.94-225.40, with an average of approximately 84.54 times. The core papers are mainly published in 2018 and 2019, with the average publication year of core papers on "intelligent identification mechanism and real time monitoring technology for crop diseases and pests", "straw modification and rapid decomposition technology", "antibiotic-free nutritional regulation techniques for the intestinal health and growth of livestock and poultry", "intelligent collaborative operation technology for multiple agricultural machinery", and "diagnosis of forest diseases and pests based on deep learning" being 2019, compared to other selected fronts, it is closer to the current situation (Tables 1.1.1 and 1.1.2).

Table 1.1.1 Top 10 engineering research fronts in agriculture

No.	Engineering research front	Core papers	Citations	Citations per paper	Mean year
1	Crop pan-genome	50	11 270	225.40	2017.7
2	Mechanisms and methods for synergistic improvement of crop yield, quality, and efficiency	43	3 684	85.67	2018.5
3	Genetic basis and regulatory network for the quality formation of horticultural crops	58	3 321	57.26	2018.6
4	Intelligent identification mechanism and real-time monitoring technology for crop diseases and pests	42	4 876	116.10	2019.2
5	Straw modification and rapid decomposition technology	67	1 939	28.94	2019.8
6	Mechanisms of host inflammatory response regulation mediated by significant animal pathogens	54	2 603	48.20	2018.3
7	Antibiotic-free nutritional regulation techniques for the intestinal health and growth of livestock and poultry	35	2 313	66.09	2019.1
8	Functional gene identification by multi-omics in animals	50	2 645	52.90	2018.2
9	Intelligent collaborative operation technology for multiple agricultural machinery	40	3 615	90.38	2019.2
10	Diagnosis of forest diseases and pests based on deep learning	18	1 340	74.44	2019.3

Table 1.1.2 Annual number of core papers published for the Top 10 engineering research fronts in agriculture

No.	Engineering research front		2018	2019	2020	2021	2022
1	Crop pan-genome		7	9	9	3	0
2	Mechanisms and methods for synergistic improvement of crop yield, quality, and efficiency		16	8	6	4	0
3	Genetic basis and regulatory network for the quality formation of horticultural crops		13	15	12	2	1
4	Intelligent identification mechanism and real-time monitoring technology for crop diseases and pests		6	10	11	7	1
5	Straw modification and rapid decomposition technology	11	7	13	5	14	17
6	Mechanisms of host inflammatory response regulation mediated by significant animal pathogens	16	18	11	5	3	1
7	Antibiotic-free nutritional regulation techniques for the intestinal health and growth of livestock and poultry	0	12	11	10	1	1
8	Functional gene identification by multi-omics in animals	15	18	9	7	0	1
9	Intelligent collaborative operation technology for multiple agricultural machinery		9	5	13	7	0
10	Diagnosis of forest diseases and pests based on deep learning	3	3	3	5	3	1

#### (1) Crop pan-genome

A pan-genome is a collection of all DNA sequences of a species. In recent years, crop pan-genomics has become an essential field of the studies in crop genomics. It plays a key role in revealing the genetic variations, evolutionary histories and functional genes in crops. Currently, all the staple crops, such as maize, rice and wheat, have been assembled with multiple high-quality reference genomes. Comparing to the traditional single reference genome for a species, it has become obvious that multiple genome assemblies of different individuals or varieties will provide more adequate and accurate information in presenting the genetic diversity of the species, especially reveal the rich structure variations, such as tandem repeats, present/absent variations and chromosome translocations. Constructing pan-genome at genus level will provide valuable evidence for understanding on crop origin and evolution, decoding the domestication and de-domestication process, and revealing the evolution process of functional genes. Moreover, integrating the resourceful sequence information provides opportunity for resolving the core genes and dispensable genes at pan-genome level, promoting the mining and exploiting of beneficial gene recourses. In recent years, the achievement of super-high-quality genome assemblies, such as gap-less genome and telomere-to-telomere complete genome assemblies provide strong guidance in constructing crop pan-genome. The methodological method and analysis approaches based on pan-genomes are unceasingly improving, and the tasks such as the sequence mapping on pan-genome, functional annotation of gene family, and constructing graph-based pan-genome are calling for technical innovations. In the future, incorporating with advanced technologies, such as artificial intelligence and machine learning, and abundant phenotype data, the crop pan-genomics is expected to serve as a uniform and comprehensive coordinate system for facilitating gene mining and molecular design breeding, ultimately accelerate the crop basic research and development of improved varieties for ensuring national food security and sustainable agricultural development.

## (2) Mechanisms and methods for synergistic improvement of crop yield, quality, and efficiency

The coordinated improvement of crop yield, quality and efficiency refers to the systematic revelation of the rules and mechanisms underlying the synergistic formation of high yield, superior quality, and high efficiency. This involves selecting

suitable varieties and optimal planting times based on local conditions to make full use of local temperature and light resources, optimizing nutrient management and irrigation to achieve efficient utilization of fertilizer and water resources. As a result, it accomplishes the synergistic enhancement of crop yield, quality, and efficiency. At present, the yield per unit area of major crops such as rice, wheat, and corn in China has reached a relatively high level. However, with the rapid development of China's economy and society as well as the continuous improvement in people's quality of life, the modern high-quality development of agriculture poses new requirements for crop production. There is an urgent need to not only focus on increasing yield per unit area but also to effectively enhance quality and resource utilization efficiency. Therefore, it is crucial to analyze the mechanisms and explore the pathways for the coordinated improvement of crop yield, quality, and efficiency. The core scientific issues in this regard mainly include:

- 1) The interplay between crop growth, development, yield, quality and efficiency, the comprehensive effects of crop-environment-cultivation measures on yield and quality efficiency, and the mechanisms underlying the coordinated improvement of crop yield and quality efficiency.
- 2) The evaluation index system and comprehensive selection methods for crop varieties that promote coordinated improvement in yield, quality and efficiency.
- 3) The regulatory pathways and key technologies that can be widely applied for the coordinated improvement of crop yield, quality and efficiency. Breakthroughs in the relevant theories and technologies mentioned above will provide essential theoretical support and modern engineering solutions for China's coordinated production of high yield, superior quality, and high efficiency crops.

## (3) Genetic basis and regulatory network for the quality formation of horticultural crops

As the horticultural industry develops rapidly and the total output of horticultural products increases significantly, changes in product quality, health component content, and quality and safety issues of horticultural products have received close attention. The quality of horticultural products includes factors such as appearance, nutritional quality, and flavor quality, mainly including organ size, shape, uniformity, color, storage and transport ability, various nutrient contents (sugar, acid, starch, vitamins, mineral nutrients, flavonoids, etc.), various flavor substance contents (amino acids, aromatic substances, etc.), and taste (softness, hardness, and flavor perception). The quality traits are complex. In recent years, some research has been carried out in China on fruit shape, color, nutritional quality, flavor, and the formation and regulation mechanism of bitter substances in horticultural crops. In particular, the use of genomics, transcriptomics, metabolomics, and other methods has clarified the key regulatory genes for a few metabolic substances by exploring and analyzing the metabolic genes and their regulatory genes related to horticultural product quality. There is also an increasing understanding of the molecular mechanisms and regulatory networks of important agronomic traits. However, many metabolic substances that determine the quality of horticultural products are still unclear, and there is limited research on the molecular mechanisms and metabolic regulatory mechanisms. Therefore, in the future, different levels of gene expression cascade regulatory mechanisms and their regulatory networks, as well as the interactive regulation between plant hormone signal transduction and quality formation, the coupling regulation mechanism between quality metabolism and the environment, and other research should be carried out based on the existing foundation. It aims to explore the control network and signal transduction mechanism of horticultural product quality formation, and provide a scientific basis for improving the quality and efficiency of the horticultural production.

## (4) Intelligent identification mechanism and real-time monitoring technology for crop diseases and pests

In the system for the integrated pest management, control decision is the core, prediction is the basis of decision-making, and monitoring is an indispensable basis for prediction and decision-making. Nowadays, timely, rapid, and accurate monitoring technology is very important in plant disease and pest control. With the integration and development of high and new technologies such as remote sensing, radar, image technology, quantitative molecular detection technology, sensor, Internet of Things technology, big data analysis and artificial intelligence in recent years, relatively obvious

progress has been made in the construction of intelligent identification, real-time monitoring and early warning technology of crop diseases and pests, which has greatly improved the timeliness and accuracy of disease and insect monitoring. The intelligent identification technology of crop diseases and pests is mainly composed of several or several modules, such as capture and lure equipment, detection or high-definition shooting equipment, real-time transmission platform, remote monitoring platform, intelligent identification and counting model or algorithm, and network client of computer or mobile phone, the monitoring of pests and diseases has been transformed from the traditional manual investigation to the integrated remote automatic real-time monitoring, identification and diagnosis of real-time collection, transmission, identification, analysis and early warning of pest and disease information. Although the accuracy or application scope of most identification and monitoring models is inevitably limited, with the further development of computer technology such as artificial intelligence and deep learning, the intelligent identification and monitoring technology of crop pests and diseases integrated with traditional methods and emerging technologies will become the main development direction of disease and pest monitoring and early warning in the future.

## (5) Straw modification and rapid decomposition technology

Straw return is important for maintaining soil fertility, improving nutrient efficiency, and slowing soil acidification. However, due to the high carbon to nitrogen ratio and the complex structure of straw components, direct straw return is not easy to decompose quickly, which may cause nitrogen deficiency, aggravate the diseases, and influence the growth of the next crop. Ectopic decomposition of straw also takes at least 2 months. Therefore, how to promote the rapid decomposition of straws is one of the urgent treatment problems in current agriculture. The process of straw decomposition to organic matter was influenced by the nature of the straw itself, soil chemical and soil microbial properties. Straw modification refers to the exogenous addition of nitrogen fertilizers, nanomaterials, chemical modifiers, enzyme preparations or microbial inoculants, and so on, which can regulate the physical, chemical, biological processes during the straw decomposition, and thus improve the decomposition efficiency of straw and product efficacy. With the rapid development of techniques such as microbiology and organic substance characterization, it can be achieved to increase the speed of in-situ or ectopic straw decomposition, improve the performance of organic products and nutrient capacity based on techniques of highly efficient microbial inoculant and straw modification, and finally establish regional precision technology for rapid decomposition of straw under different climatic environments. This is of great significance for realizing the sustainable and low-carbon development of agriculture, and accelerating the agricultural recycling economy.

## (6) Mechanisms of host inflammatory response regulation mediated by significant animal pathogens

As China is the world's largest consumer of livestock and poultry and animal-derived food, the prevention and control of significant animal diseases are crucial for the healthy development of animal husbandry and public health security. The invasion of animal pathogens induces the host immune response, and the regulation of host inflammatory response is closely related to the occurrence and development of diseases. Inflammatory response is a self-defense mechanism of the host against stimuli, playing an important role in pathogen clearance, enhancing disease resistance, and preventing the spread of pathogens. Specifically, upon invading the host, pathogens are recognized by host pattern recognition receptors, initiating a series of intracellular signal transduction, recruiting a large number of chemokines, cytokines, and activated immune cells to gather at the infected site to clear the pathogens, causing local inflammatory reactions. Inflammatory response is mediated by pro-inflammatory cytokines, and some important pathogens can induce a large release of pro-inflammatory cytokines to cause "cytokine storm", leading to strong local inflammation of tissues and assisting pathogen proliferation; meanwhile, pathogens can cause macrophage lysis by activating the inflammasome, resulting in the escape and spread of pathogens. In addition, pathogens can inhibit the expression and release of pro-inflammatory factor by regulating various signaling pathways in host cells, thereby avoiding clearance by the host and establishing persistent infection. Elaborating the key mechanisms by which pathogens regulate host inflammatory response is crucial for understanding the pathogenicity of pathogens and seeking new treatment of infectious diseases. The relevant research results will also provide important guidance and theoretical basis for vaccine development and the prevention and control of significant animal diseases.

## (7) Antibiotic-free nutritional regulation techniques for the intestinal health and growth of livestock and poultry

The problems of antibiotic resistance, ecological environment pollution and food safety caused by the abuse of antibiotics have seriously affected the healthy development of animal husbandry in China. In 2020, China issued a "prohibition order" to completely prohibit the addition of growth-promoting antibiotics in feed, which brings great challenges to intestinal health and growth and development of livestock and poultry. Based on this, the development and application of green and safe nonresistant nutrition control technology to maintain the intestinal health of livestock and poultry and promote their growth and development is an inevitable trend of efficient and sustainable development of animal husbandry in China. The nonresistant nutrition regulation technology is based on the balance of feed "nutrient structure", integrating integrated nutrients to the comprehensive nutritional regulation of animal immunity, intestinal health and pathogenic factors, so as to promote the intestinal development of animals under the condition of non-resistance, improve the body's resistance to disease, and ensure animal health and efficient production. The key of non-resistant nutrition technology system lies in the balance and optimization of feed nutrient structure, feed processing modulation and precise feeding, regulating intestinal microecological balance from the aspects of nutrient source selection, formula design and optimization, additive combination screening, feed processing modulation, feeding methods and fecal bacteria transplantation, so as to promote intestinal health and efficient growth of livestock and poultry. It is difficult and hot to further strengthen the basic theoretical research of non-resistant nutrition and promote the application of non-resistant nutrition regulation technology system. The relevant research results will certainly provide theoretical guidance and technical support for the intestinal health and efficient growth of livestock and poultry under non-resistant conditions.

## (8) Functional gene identification by multi-omics in animals

Functional gene identification by multi-omics in animals is a process that involves the comprehensive analysis of various genetic, transcriptomic, and proteomic data, along with related bioinformatics methods, to identify and study genes in the animal genome that have important functions and biological significance. The process of functional gene identification in animal multi-omics typically includes the following steps: data collection, data preprocessing, bioinformatics analysis (which involves the application of bioinformatics tools and algorithms to analyze the preprocessed data, including genome assembly, gene localization, gene function annotation, transcriptome assembly, expression analysis, protein identification and quantification), functional gene mining (which involves the identification of genes with important functions and biological significance by combining the analysis results with existing biological knowledge, and may include enrichment analysis, differential expression analysis, proteinprotein interaction network analysis, etc.), functional annotation and interpretation, and biological validation (which includes experimental validation or further functional studies to confirm the important roles of functional genes in biological processes). This technique aims to decipher the molecular genetic basis of important traits such as meat quality and quantity, milk production and quality, wool production and quality, egg production, as well as growth, development, reproduction, disease resistance, heat stress resistance, and low-oxygen tolerance in superior animal breeds. It provides a basis for the theory and technological innovation of genome-guided breeding, greatly improves the accuracy of selecting and creating superior traits such as high yield, good quality, disease resistance, and stress tolerance in agricultural animals, and accelerates the process of targeted breeding of improved varieties.

#### (9) Intelligent collaborative operation technology for multiple agricultural machinery

According to statistics, the global population is projected to reach 9.7 billion by 2050, with a growth in food demand exceeding 70%. Labor shortages have become a pressing issue in agricultural production, necessitating the advancement of agricultural modernization and industrial upgrading. Unmanned intelligent agricultural machinery, through automation technology, can operate autonomously to reduce reliance on manual labor and achieve high-efficiency agricultural operations, thereby enhancing production efficiency. However, as agricultural modernization progresses, the expansion of farmland continues, and achieving intelligent and unmanned operation for a single agricultural machine may not suffice to meet the demands of large-scale, efficient production. The realization of intelligent collaborative operations among multiple agricultural machines has become a research focal point. This includes communication and networking technologies facilitating real-time data transmission and information

exchange between machines, technologies for perceiving and identifying real-time agricultural environmental conditions and crop growth statuses, autonomous planning and coordinated control technologies for multiple agricultural machines, task coordination and operation scheduling technologies among these machines, safety and security technologies for both single and multiple agricultural machine collaborative operations—covering aspects such as safe maneuvering, data security, system reliability, and fault diagnosis, among others. Breaking through the core key technologies related to these aspects will enable task specialization and collaboration in agricultural production, leading to improved operational efficiency and supporting the development of smart agricultural applications.

#### (10) Diagnosis of forest diseases and pests based on deep learning

Deep learning-based approaches for forest pest and disease diagnostics represent an innovative strategy for detecting and monitoring these threats in woodland environments. Deep learning, a subset of machine learning techniques, centers on constructing and training multi-layered neural networks to autonomously glean features and patterns from extensive datasets. Such methods excel in accurately recognizing pests and diseases and scrutinizing their prevalence trends by synergizing data. This approach offers several advantages over conventional methods of identification, including alleviating the repetitive tasks undertaken by professionals, conserving labor, providing timely insights, and delivering high levels of precision and efficiency. Current research in the realm of forest pest and disease diagnosis utilizing deep learning encompasses various dimensions. These encompass image recognition for identifying forest pests and diseases, tracking pest populations and their dynamics via the detection of light- or pheromone-induced responses. Furthermore, this approach extends to monitoring alterations in the prevalence of forest pests and diseases and predicting their trajectory using high-resolution imagery from satellites and unmanned aerial vehicles. These images encompass visible, multispectral, and hyperspectral data. As artificial intelligence technology continues to advance, constructing a comprehensive model for forest pests and diseases, delving into data fusion and multimodal information, exploring model design, compression, and automated architecture searches are imperative. These measures collectively enhance the efficiency and adaptability of the model. Predicting pest and disease occurrences based on regional monitoring networks is pivotal in adapting to contemporary environmental shifts and combating the invasion of pests and diseases. This is particularly vital for ensuring the sustainable development of forestry under prevailing mega-trends.

## 1.2 Interpretations for three key engineering research fronts

#### 1.2.1 Genetic basis and regulatory network for the quality formation of horticultural crops

With the rapid development of the horticulture industry and the substantial increase in total production of horticultural crops, there has been a growing concern for changes in product quality characteristics, content of health-promoting compounds, and the safety of horticultural products. Horticultural product quality includes aspects such as appearance, nutritional quality, and flavor quality, which mainly involve organ size, shape, uniformity, color, shelf-life, content of various nutrients (sugar, acid, starch, vitamins, mineral nutrients, flavonoids, etc.), content of various flavor compounds (amino acids, aromatic substances, etc.), and sensory attributes (texture, mouthfeel, etc.). The characteristics of product quality are complex. In recent years, China has conducted research on fruit shape, color, nutritional quality, flavor, and the formation and regulation mechanisms of bitter compounds in horticultural crops. Particularly, methods such as genomics, transcriptomics, and metabolomics have been applied to identify and analyze genes related to the metabolism of substances affecting horticultural product quality. This research has shed light on the key regulatory genes for a small number of metabolites and has led to a better understanding of the molecular mechanisms and regulatory networks underlying important agronomic traits. However, the molecular mechanisms and metabolic regulation of most metabolites that determine horticultural product quality remain unclear, with limited research in this area.

With the rapid development of sequencing technology and the quick decrease in costs, the genomes of many horticultural plants have been sequenced, or updated to more high-quality genomes. Chinese scientists have made outstanding contributions in this field. According to incomplete statistics, genome sequencing has been completed for more than 50 horticultural crops, providing a foundation for breeding new and superior crop varieties through molecular design methods.

Currently, over 60 quantitative trait loci controlling sugar content have been identified. Additionally, proteins indirectly involved in sugar metabolism, such as sucrose transporters, starch synthesis enzymes, and vacuole processing enzymes, have been analyzed. The team led by YE Zhibiao successfully identified major-effect locus TFM6 (tomato fruit malate 6), an Al-activated malate transporter ALMT9, that regulates malate accumulation in tomatoes through association analysis (mGWAS). They confirmed that WRKY42 negatively regulates ALMT9 expression by binding to the W-box on the ALMT9 promoter, thereby inhibiting malate accumulation in tomato fruits. It has been reported that 237 loci in tomatoes are associated with enzyme-catalyzed reactions in the ascorbic acid metabolic pathway, and the metabolic network of genes and corresponding enzymes related to the ascorbic acid metabolic pathway has been preliminarily established.

Many genes involved in carotenoid biosynthesis pathways have been isolated, cloned, and functionally validated. Secondary metabolites such as flavonoids, alkaloids, phenylpropanoids, and aromatic compounds are also important components in the formation of nutritional and flavor quality in horticultural crops. In this field, China's Institute of Agricultural Sciences and the University of Florida in the USA have collaborated to identify 33 key flavor compounds influencing consumer preferences. They have also discovered 49 key loci regulating the accumulation of flavor compounds, revealing the material basis and genetic improvement path of tomato flavor. This provides feasible breeding solutions for cultivating delicious tomatoes.

Due to the complexity of quality traits, research on the genetic basis and regulatory networks underlying trait formation is still in its early stages. The future focus of research will be on the application of molecular biology techniques to establish and optimize genetic transformation systems for horticultural crops, to uncover key genes related to crop quality, to elucidate the regulatory role of these genes in quality, and to establish a more precise molecular regulatory network by identifying important nodal regulatory genes. Efforts will be made to break the unfavorable gene linkage with quality traits, achieve simultaneous improvements in horticultural crop quality and other desirable traits such as stress resistance, and breed high-quality horticultural crop varieties. The mechanisms by which environmental factors and cultivation techniques regulate quality will be explored, and key technologies for quality horticultural crop cultivation in controlled environments will be established.

In the front of "genetic basis and regulatory network for the quality formation of horticultural crops", the top three countries in terms of the publication of core papers (Table 1.2.1) are China (63.79%), the USA (27.59%), and Italy (12.07%). The citations per paper in this front is distributed from 46.25 to 93.67, of which the citations per paper of Israel and the USA exceeds 70.00. In terms of the distribution of research institutions (Table 1.2.2), Chinese Academy of Sciences (CAS), Anhui Agricultural University (AAU), and Chinese Academy of Agricultural Sciences (CAAS) have produced many core papers and cited many times. Cooperation among countries is more common, and cooperation among China and the USA is relatively closer (Figure 1.2.1). The output is mainly in the cooperation network among institutions (Figure 1.2.2), and there are certain cooperation relations among these institutions. The main output countries of the citing papers are China and the USA, with China accounting for 49.05% and the USA accounting for 14.35% (Table 1.2.3). In terms of the main output institutions of the citing papers (Table 1.2.4), CAS, CAAS, and Nanjing Agricultural University (NAU) ranked among the top three in the number of citing papers. Figure 1.2.3 shows the roadmap of the engineering research front of "genetic basis and regulatory network for the quality formation of horticultural crops".

Table 1.2.1 Countries with the greatest output of core papers on "genetic basis and regulatory network for the quality formation of horticultural crops"

No.	Country	Core papers	Percentage of core papers/%	Citations	Citations per paper	Mean year
1	China	37	63.79	2 288	61.84	2018.7
2	USA	16	27.59	1 178	73.62	2019.1
3	Italy	7	12.07	423	60.43	2017.7
4	UK	7	12.07	367	52.43	2019.1
5	Israel	6	10.34	562	93.67	2018.2
6	Spain	6	10.34	281	46.83	2018.5
7	France	6	10.34	279	46.50	2018.5
8	Netherlands	5	8.62	261	52.20	2019.2
9	Austria	4	6.90	185	46.25	2018.0
10	Germany	3	5.17	160	53.33	2018.7

Table 1.2.2 Institutions with the greatest output of core papers on "genetic basis and regulatory network for the quality formation of horticultural crops"

No.	Institution	Core papers	Percentage of core papers/%	Citations	Citations per paper	Mean year
1	Chinese Academy of Sciences	6	10.34	384	64.00	2019.7
2	Anhui Agricultural University	6	10.34	302	50.33	2018.7
3	Chinese Academy of Agricultural Sciences	5	8.62	280	56.00	2020.0
4	Huazhong Agricultural University	4	6.90	342	85.50	2017.5
5	Zhejiang University	4	6.90	329	82.25	2018.8
6	University of California, Davis	4	6.90	251	62.75	2019.2
7	Edmund Machinery Foundation Research and Innovation Center	4	6.90	218	54.50	2017.8
8	South China Agricultural University	4	6.90	190	47.50	2018.8
9	Cornell University	3	5.17	387	129.00	2018.3
10	United States Department of Agriculture Agricultural Research Service	3	5.17	266	88.67	2019.3

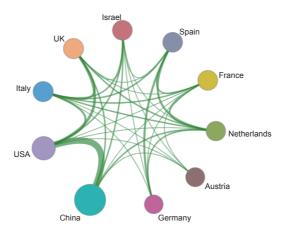


Figure 1.2.1 Collaboration network among major countries in the engineering research front of "genetic basis and regulatory network for the quality formation of horticultural crops"

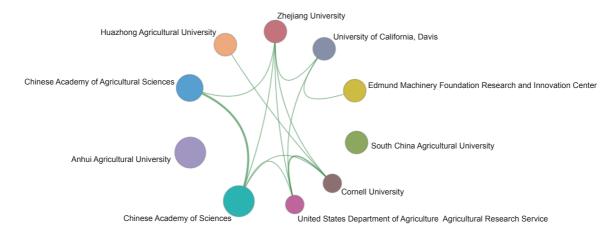


Figure 1.2.2 Collaboration network among major institutions in the engineering research front of "genetic basis and regulatory network for the quality formation of horticultural crops"

Table 1.2.3 Countries with the greatest output of citing papers on "genetic basis and regulatory network for the quality formation of horticultural crops"

No.	Country	Citing papers	Percentage of citing papers/%	Mean year
1	China	1 623	49.05	2021.1
2	USA	475	14.35	2020.8
3	Italy	216	6.53	2020.7
4	Spain	174	5.26	2020.8
5	Germany	161	4.87	2020.9
6	India	155	4.68	2021.2
7	France	154	4.65	2020.5
8	Britain	110	3.32	2021.0
9	Australia	84	2.54	2020.5
10	Japan	79	2.39	2020.8

Table 1.2.4 Institutions with the greatest output of citing papers on "genetic basis and regulatory network for the quality formation of horticultural crops"

No.	Institution	Citing papers	Percentage of citing papers/%	Mean year
1	Chinese Academy of Sciences	150	15.59	2021.1
2	Chinese Academy of Agricultural Sciences	141	14.66	2021.1
3	Nanjing Agricultural University	100	10.40	2021.0
4	South China Agricultural University	92	9.56	2021.0
5	Huazhong Agricultural University	90	9.36	2020.9
6	Zhejiang University	73	7.59	2021.1
7	Fujian Agriculture and Forestry University	70	7.28	2021.1
8	Anhui Agricultural University	70	7.28	2020.8
9	United States Department of Agriculture Agricultural Research Service	62	6.44	2020.8
10	Shandong Agricultural University	57	5.93	2021.2

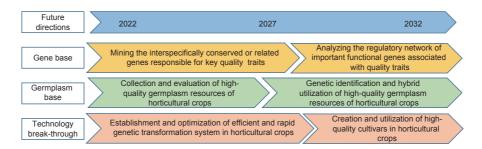


Figure 1.2.3 Roadmap of the engineering research front of "genetic basis and regulatory network for the quality formation of horticultural crops"

#### 1.2.2 Functional gene identification by multi-omics in animals

The healthy development of the livestock and poultry industry is a strategic necessity to ensure the safety of our country's grain and livestock products. As a key component of the breeding industry, the seed industry will further contribute to the development of animal husbandry in the future. The molecular mechanisms underlying important economic traits in livestock and poultry are extremely complex. Currently, the incomplete annotation of functional genomic maps and the unclear understanding of key genes and regulatory mechanisms for excellent traits severely restrict the efficient utilization of germplasm resources, genetic improvement, and the creation of new breeds in livestock and poultry.

With the continuous development of modern biotechnology, livestock and poultry breeders have progressed from macroscopically describing the genetic laws of traits based on genetic power to analyzing the regulatory regions that influence quantitative traits using molecular markers, and now to directly deciphering the relationship between DNA sequence variations and complex traits. These known causative mutations of important traits can be directly applied to breeding practices, laying a solid foundation for the cultivation of superior breeds. Functional gene mining in animal multiomics is crucial for deciphering the molecular genetic basis of important economic traits in livestock and poultry, as well as for genome sequencing and functional genomics research. It is also an important prerequisite for the development of superior animal breeds and has been a focus of research in the field of animal genetic breeding both domestically and internationally for a long time.

Since 2009, whole-genome selection technology based on genome-wide SNP chips has rapidly developed and been widely used in dairy cattle, beef cattle, pigs, and broilers breeding in developed countries in Europe and America. In 2014, Australian scientists initiated the international 1 000 Bull Genomes Project. In 2015, the European Union and the USA established the "International Animal Genomic Functional Annotation Project" to predict the important traits of livestock and poultry germplasm resources. Chinese scientists have led or participated in genome projects of different livestock and poultry species such as chickens, ducks, pigs, geese, yaks, and sheep. They have also jointly launched scientific programs like the Ten Thousand Bird Genomes Project and the Macro-genome Project with different countries, laying a foundation for elucidating the genetic mechanisms of important economic traits. In addition, domestic and foreign researchers have identified a group of differentially expressed genes, differentially expressed proteins, and differentially methylated genes related to important economic traits such as growth, meat quality, reproduction, and milk production. They have explored the regulatory relationships between them and made important discoveries in molecular mechanisms such as pig carcass traits, milk composition synthesis and mastitis resistance in dairy cows, and the development of Cashmere goat hair follicles. They have obtained whole-genome copy number variation maps for cattle, pigs, beef cattle, chickens, and ducks, and discovered multiple candidate genes related to milk production traits, body size traits, carcass traits, immune traits, and meat quality traits. The initiation of domestic and international animal genomic functional annotation projects (FAANG, FarmGTEx, etc.) has led to the elucidation of the molecular regulatory mechanisms of agricultural animal phenotypic traits

in the era of genomics. Meanwhile, as functional gene mining in animal multi-omics continues to deepen in basic research applications, agricultural animal breeding is accelerating its transition from traditional conventional breeding to multi-omics intelligent design breeding.

Animal multi-omics functional gene mining technology mainly includes comprehensive analysis of big data in animal pangenomics, genomics, epigenomics, spatiotemporal transcriptomics, proteomics, metabolomics, and phenomics. It integrates methods from genetics, genomics, bioinformatics, molecular biology, biochemistry, cell biology, and animal breeding to reveal panoramic multi-dimensional omics features of animals. It aims to identify genetic loci and molecular markers (QTL) related to traits such as high productivity, high quality, stress resistance, and disease resistance in animals. It explores key genetic variations, functional genes, regulatory sequences, and regulatory networks, revealing the interactions between genes, phenotypes, and the environment. It provides molecular genetic selection markers and manipulation targets for animal breeding. This technology aims to decipher the molecular genetic basis of important traits such as meat quantity and quality, milk yield and dairy quality, fur yield and quality, egg production, as well as growth, development, reproduction, disease resistance, heat stress tolerance, and other important traits in excellent animal germplasm. It provides a basis for genomeguided precision breeding theory and technological innovation, greatly improving the accuracy of selecting and creating high-yielding, high-quality, disease-resistant, and stress-tolerant traits in agricultural animals, and accelerating the targeted breeding process of superior breeds.

In terms of core papers published in the front of "functional gene identification by multi-omics in animals", the top three countries are the USA (38.00%), China (38.00%), and the UK (10.00%) (Table 1.2.5). The citations per paper of this front ranges from 45.00 to 82.00, with the UK, Australia, and the Netherlands are all more than 65.00. In terms of the main output institutions, University of California, Davis, University of Toulouse, and Northwest A & F University produce core papers with higher citations per paper (Table 1.2.6). As shown in Figure 1.2.4, research cooperation between countries is relatively common, with the cooperation between the UK, the USA, and China being relatively closer. As shown in Figure 1.2.5, there is a certain degree of cooperation among main institutions. The top two countries in the number of citing papers are China and the USA, with China accounting for 30.38% and the USA accounting for 24.14%, and the mean year is relatively late, demonstrating strong research and development momentum (Table 1.2.7). In terms of the main institutions of citing papers (Table 1.2.8), CAS, CAAS, and China Agricultural University rank top three,

Table 1.2.5 Countries with the greatest output of core papers on "functional gene identification by multi-omics in animals"

No.	Country	Core papers	Percentage of core papers/%	Citations	Citations per paper	Mean year
1	USA	19	38.00	1 153	60.68	2018.6
2	China	19	38.00	924	48.63	2018.4
3	UK	5	10.00	348	69.60	2018.6
4	France	5	10.00	287	57.40	2018.0
5	Germany	5	10.00	225	45.00	2018.4
6	Australia	4	8.00	288	72.00	2018.5
7	Canada	4	8.00	235	58.75	2017.8
8	Denmark	3	6.00	168	56.00	2018.0
9	Spain	3	6.00	167	55.67	2018.0
10	Netherlands	2	4.00	164	82.00	2018.5

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Table 1.2.6 Institutions with the a	ireatest output of core papers on	Tunctional dene identification b	v muiti-omics in animais

No.	Institution	Core papers	Percentage of core papers/%	Citations	Citations per paper	Mean year
1	University of California, Davis	4	8.00	270	67.50	2018.5
2	University of Toulouse	4	8.00	252	63.00	2018.2
3	Northwest A & F University	4	8.00	190	47.50	2018.0
4	The University of Queensland	3	6.00	232	77.33	2018.3
5	University of Edinburgh	3	6.00	224	74.67	2018.7
6	Iowa State University	3	6.00	205	68.33	2020.0
7	United States Department of Agriculture Agricultural Research Service	3	6.00	190	63.33	2019.0
8	Aarhus University	3	6.00	168	56.00	2018.0
9	National Research Institute for Agriculture, Food and the Environment	3	6.00	163	54.33	2017.7
10	China Agricultural University	3	6.00	159	53.00	2019.3

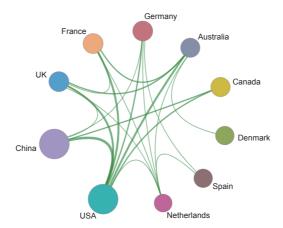


Figure 1.2.4 Collaboration network among major countries in the engineering research front of "functional gene identification by multi-omics in animals"

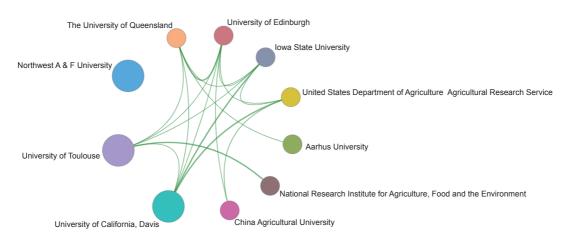


Figure 1.2.5 Collaboration network among major institutions in the engineering research front of "functional gene identification by multi-omics in animals"

Table 1.2.7 Countries with the greatest output of citing papers on "functional gene identification by multi-omics in animals"

No.	Country	Citing papers	Percentage of citing papers/%	Mean year
1	China	979	30.38	2021.0
2	USA	778	24.14	2020.5
3	UK	247	7.66	2020.5
4	Germany	235	7.29	2020.3
5	France	177	5.49	2020.5
6	Australia	172	5.34	2020.6
7	Canada	145	4.50	2020.5
8	India	144	4.47	2020.9
9	Italy	127	3.94	2020.4
10	Spain	123	3.82	2020.5

Table 1.2.8 Institutions with the greatest output of citing papers on "functional gene identification by multi-omics in animals"

No.	Institution	Citing papers	Percentage of citing papers/%	Mean year
1	Chinese Academy of Sciences	104	14.05	2021.1
2	Chinese Academy of Agricultural Sciences	102	13.78	2020.9
3	China Agricultural University	86	11.62	2020.7
4	University of Edinburgh	81	10.95	2020.7
5	United States Department of Agriculture Agricultural Research Service	75	10.14	2020.7
6	Northwest A & F University	59	7.97	2020.7
7	Huazhong Agricultural University	56	7.57	2021.1
8	The University of Queensland	52	7.03	2020.4
9	Sichuan Agricultural University	47	6.35	2020.7
10	University of California, Davis	40	5.41	2020.5

with the number of citing papers of CAS ranking first.

It is worth noting that complex economic traits in animals are governed by a large number of genetic components, which are exhibited through intricate developmental regulatory networks and gradually shaped by natural or artificial selection. The contribution of a single gene to a trait is limited, and its effect can vary significantly under different environmental conditions. The regulatory networks that involve coupling and antagonism between different traits, as well as their underlying core regulatory units, have not been fully deciphered. The phenomena of multiple effects caused by genetic linkage and one effect caused by multiple factors have become key bottlenecks in animal multi-omics functional gene mining. These bottlenecks also limit molecular design breeding in animals, especially the genetic progress in multi-trait coordinated improvement. Therefore, future research directions should focus on two aspects: enriching the sources of multi-omics data and expanding the depth of multi-omics integrated analysis. ① Traditional functional genomics studies that focus on static genetic associations for single traits (such

as SNP and GWAS), or functional studies of individual genes or signaling pathways during development, have difficulty accurately depicting the systemic evolutionary framework from genotype to phenotype. To address the unique multi-factor regulatory models underlying different economic traits, emerging integrated analysis technologies such as three-dimensional genomics (Hi-C), protein interaction networks, multi-dimensional epigenomics (ATAC-Seq, ChIP-seq), single-cell sequencing, spatial transcriptomics, gene editing techniques, and optimized GWAS algorithms can be fully utilized. These technologies can help unravel the regulatory networks formed by the combination and interaction of different alleles at different loci, which contribute to the formation of complex trait phenotypes. Key functional elements that can be used in breeding practices can be discovered.

② Molecular genetic studies of important traits in agricultural animals require the support of large-scale experimental populations and data, as well as continuous, systematic, and in-depth monitoring and research on key phenotypes. Therefore, the extensive research on multi-omics intelligent design breeding inevitably leads to an exponential growth of multi-omics information in animals. Interpreting massive and heterogeneous multi-omics data and integrating multi-omics information from different studies to analyze the relationship between genetic variations and important economic traits present significant challenges. Overcoming the bottleneck of single-omics analysis limited to "correlation" between markers and phenotypes and breaking through the difficulties in revealing "causality" can be achieved through reference to animal multi-omics databases and dynamic integrated analysis methods. From an integrative and systems biology perspective, the genetic basis of complex traits in animals can be

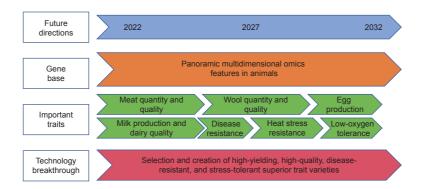


Figure 1.2.6 Roadmap of the engineering research front of "functional gene identification by multi-omics in animals" elucidated. Figure 1.2.6 shows the roadmap of the engineering research front of "functional gene identification by multi-omics in animals".

## 1.2.3 Diagnosis of forest diseases and pests based on deep learning

Forests, as intricate non-rigid organic entities within nature, play a pivotal role in ecosystems by shaping ecological niches, facilitating energy flow, and orchestrating material cycling. Furthermore, they stand as vital carbon sinks, harboring one of the largest repositories of organic carbon within terrestrial ecosystems. A report from the United Nations' Food and Agriculture Organization (FAO) indicated that in 2020, global forest coverage spanned 4.045 billion hectares, constituting 31% of the Earth's land area. Pairing this with insights from China's comprehensive forest resources assessments and statistical data, the calculation of average forest benefits reveals that China's per-unit forest area annually retains 192.34 tons of water, absorbs 12.1 kilograms of airborne pollutants, and releases 315 kilograms of oxygen. However, challenges persist, particularly concerning the health of forest ecosystems. In 2022, China encountered substantial threats from forestry pests, with 178 million mu (approximately 11.87 million hectares) of forest land imperiled by major pests. Pine wood nematode disease affected 22 million mu (approximately 1.47 million hectares) of forest land, resulting in 10 million diseased and dead pine trees. Additional threats, such as the white moth, imperiled another 28 million mu (approximately 1.87 million hectares). Consequently, research into diagnosing forest tree diseases and pests holds immense significance for upholding ecosystem health, enhancing forestry productivity, and safeguarding biodiversity.

Traditional methods of pest and disease identification in forests rely on manual techniques and conventional image processing methods. These approaches often suffer from limitations related to human judgment, constrained feature extraction capabilities,

and compromised accuracy and efficiency. A scarcity of skilled technicians further exacerbates these issues, leading to substantial economic losses caused by delayed prevention and control efforts or excessive intervention that impacts ecological security and biodiversity.

Deep learning-based methods, on the other hand, capitalize on constructing intricate neural networks to autonomously grasp complex features and patterns from extensive datasets, resulting in more precise and rapid pest and disease diagnoses. These methods encompass a spectrum of algorithms, including multi-task learning for versatile diagnosis, transfer learning to minimize data requirements and training time, target detection algorithms to pinpoint pest and disease locations, and semantic segmentation to precisely delineate infected and healthy areas. Ongoing research within the domain of deep learning-based forest pest and disease diagnostics centers on several key facets. These include bolstering model efficiency and generalization, enabling real-time monitoring and feedback, enhancing automation and intelligence, leveraging data fusion and multimodal information, and harnessing the potential of Al's large-scale models. This approach chiefly concentrates on areas such as forest pest and disease image recognition, population dynamics monitoring through techniques like lamp lure or pheromone-induced pest counting, trend monitoring in forest pest and disease areas using satellite imagery and unmanned aerial vehicle data (encompassing visible, multispectral, and hyperspectral information), and the prediction of pest and disease occurrence trends.

In the front of "diagnosis of forest diseases and pests based on deep learning", the three leading countries in terms of the number of core papers published (Table 1.2.9) are the USA, China, and India. The citations per paper of this front ranges from 45.00 to 123.00, with the exceptions of Egypt and India, which exhibit a citation frequency surpassing 50.00. In terms of research institution distribution (Table 1.2.10), institutions such as COMSATS University Islamabad, Prince Sultan University, University of Washington, University of Hawaii, and University of Hawaii at Manoa have demonstrated a high output of core papers along with a high citations per paper. The primary countries contributing to the citing papers include China, India, and the USA (Table 1.2.11). Regarding the main institutions of citing papers (Table 1.2.12), CAS, COMSATS University Islamabad, and Deakin University hold prominent positions in terms of the number of citing papers. Collaboration between countries is extensive and interconnected (Figure 1.2.7), with relatively close collaborations observed between Egypt, India, and Saudi Arabia. As for the collaborative network among the main institutions (Figure 1.2.8), there exists a degree of cooperation both within institutions of the same country and across institutions in different countries. For instance, notable collaboration exists between Seoul National University and University of Waterloo, University of Hawaii and University of Hawaii at Manoa, and University of Washington, Prince Sultan University, and

Table 1.2.9 Countries with the greatest output of core papers on "diagnosis of forest diseases and pests based on deep learning"

No.	Country	Core papers	Percentage of core papers/%	Citations	Citations per paper/%	Mean year
1	USA	5	27.78	436	87.20	2019.4
2	China	5	27.78	263	52.60	2019.4
3	India	4	22.22	196	49.00	2020.2
4	Saudi Arabia	3	16.67	249	83.00	2021.0
5	Pakistan	2	11.11	219	109.50	2020.0
6	Australia	2	11.11	124	62.00	2019.0
7	Brazil	2	11.11	105	52.50	2020.0
8	Egypt	2	11.11	90	45.00	2020.0
9	Canada	1	5.56	123	123.00	2017.0
10	Republic of Korea	1	5.56	123	123.00	2017.0

Table 1.2.10 Institutions with the greatest output of core papers on "diagnosis of forest diseases and pests based on deep learning"

No.	Institution	Core papers	Percentage of core papers/%	Citations	Citations per paper/%	Mean year
1	China University of Mining and Technology (Beijing)	2	11.11	122	61.00	2019.5
2	COMSATS University Islamabad	1	5.56	150	150.00	2020.0
3	Prince Sultan University	1	5.56	150	150.00	2020.0
4	University of Washington	1	5.56	150	150.00	2020.0
5	University of Hawaii	1	5.56	131	131.00	2018.0
6	University of Hawaii at Manoa	1	5.56	131	131.00	2018.0
7	Seoul National University	1	5.56	123	123.00	2017.0
8	University of Waterloo	1	5.56	123	123.00	2017.0
9	Yonsei University	1	5.56	123	123.00	2017.0
10	Emory University	1	5.56	123	123.00	2021.0

Table 1.2.11 Countries with the greatest output of citing papers on "diagnosis of forest diseases and pests based on deep learning"

No.	Country	Citing papers	Percentage of citing papers/%	Mean year
1	China	395	28.66	2021.2
2	India	217	15.75	2021.5
3	USA	215	15.60	2020.9
4	Saudi Arabia	109	7.91	2021.5
5	UK	78	5.66	2021.1
6	Republic of Korea	77	5.59	2021.1
7	Pakistan	76	5.52	2021.4
8	Australia	62	4.50	2020.7
9	Italy	55	3.99	2020.9
10	Spain	47	3.41	2021.4

Table 1.2.12 Institutions with the greatest output of citing papers on "diagnosis of forest diseases and pests based on deep learning"

No.	Institution	Citing papers	Percentage of citing papers/%	Mean year
1	Chinese Academy of Sciences	34	17.62	2021.4
2	COMSATS University Islamabad	25	12.95	2021.3
3	Deakin University	17	8.81	2019.3
4	Prince Sultan University	16	8.29	2021.0
5	Northwestern Polytechnic University	16	8.29	2021.1
6	University of Washington	15	7.77	2021.9
7	Noroff University College	14	7.25	2021.9
8	King Abdullah University of Science & Technology	14	7.25	2021.9
9	King Saud University	14	7.25	2021.8
10	Central South University	14	7.25	2021.4

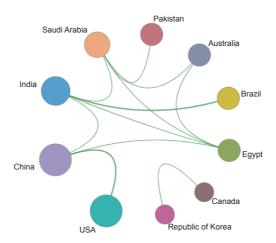


Figure 1.2.7 Collaboration network among major countries in the engineering research front of "diagnosis of forest diseases and pests based on deep learning"

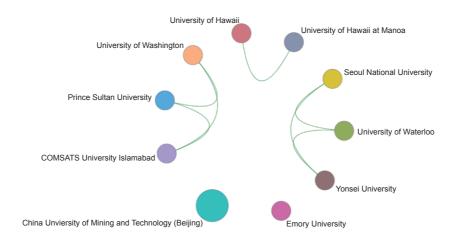


Figure 1.2.8 Collaboration network among major institutions in the engineering research front of "diagnosis of forest diseases and pests based on deep learning"

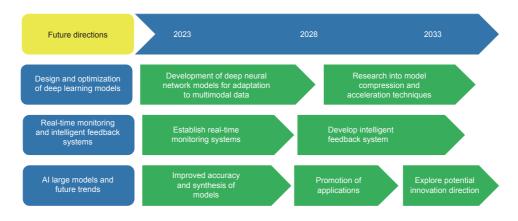


Figure 1.2.9 Roadmap of the engineering research front of "diagnosis of forest diseases and pests based on deep learning"

COMSATS University Islamabad. Figure 1.2.9 shows the roadmap of the engineering research front of "diagnosis of forest diseases and pests based on deep learning".

# 2 Engineering development fronts

## 2.1 Trends in Top 11 engineering development fronts

The Top 11 engineering development fronts in the agricultural field mainly involves directions such as agricultural green development, smart agriculture, and agricultural engineering, and reflects interdisciplinary applications (Table 2.1.1). Among them, the engineering development fronts related to agricultural green development include "crop green super-high-yield cultivation technology", "development and utilization of high-quality germplasm resources for horticultural crops", "molecular design of green pesticides based on structural biology", "synergistic technology for efficient conversion of organic matter and reduction of pollutants during composting", and "bio-refinery of wood waste". At the same time, the in-depth application of gene editing technology is a hot research topic for researchers, such as "key technologies for unmanned farms" and "ecological breeding technology of aquatic animals". The disclosure of core patents related to various cutting-edge technologies from 2017 to 2022 is shown in Table 2.1.2. Among them, the citations per patent of "molecular design of green pesticides based on structural biology" is as high as 19.61, indicating that agricultural green development has received widespread attention from researchers in recent years. The core patents for "development and application of genome editors in crops" have the highest number of disclosures, with 4 658 published patents (up to 1 292 in 2022) and 63 027 citations, far higher than other development fronts. The core patents for "creation of novel and efficient animal vaccines" are the least, and there were no core patents in 2022.

#### (1) Development and application of genome editors in crops

Key technologies for unmanned farms

Bio-refinery of wood waste

Ecological breeding technology of aquatic animals

Genome editing refers to the genetic manipulation technology of precise insertion, deletion or replacement of DNA. Through precise editing of functional genes or regulatory sequences in organisms, genome editing technology is able to repair target genes, strengthen beneficial genes, eliminate harmful genes, and ultimately achieve the purpose of improving the function of organisms. As the most subversive and leading cutting-edge technology of biological breeding, genome editing technology has become the priority in developed countries and multinational seed companies, and is the competition commanding height in the field of global agricultural biotechnology. Using genome editing technology, Chinese scientists have realized the genetic improvement

**Published** Citations per Mean year Engineering development front Citations patent patents 1 Development and application of genome editors in crops 2020.3 4 658 63 027 13.53 Crop green super-high-yield cultivation technology 2019.7 2 181 437 2.41 Development and utilization of high-quality germplasm resources for 3 88 1.56 2020.3 137 horticultural crops 4 Molecular design of green pesticides based on structural biology 111 2 177 19.61 2018.8 Synergistic technology for efficient conversion of organic matter and 5 797 8.76 2018.9 6 983 reduction of pollutants during composting 6 Creation of novel and efficient animal vaccines 119 2.29 2019.0 52 7 Preparation of feed by pre-digestion fermentation bioprocessing 73 76 1.04 2019.3 8 Genomic mating breeding technology for livestock and poultry 856 1 587 1.85 2020.0

1 000

46

52

3 541

71

25

3.54

1.54

0.48

2019.7

2019.5

2020.6

Table 2.1.1 Top 11 engineering development fronts in agriculture

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Table 2.1.2 Annual number of core patents published for the Top 11 engineering development fronts in agriculture

No.	Engineering development front	2017	2018	2019	2020	2021	2022
1	Development and application of genome editors in crops	281	455	695	807	1 128	1 292
2	Crop green super-high-yield cultivation technology	20	24	43	23	39	32
3	Development and utilization of high-quality germplasm resources for horticultural crops		10	7	7	26	28
4	Molecular design of green pesticides based on structural biology	36	18	20	14	11	12
5	Synergistic technology for efficient conversion of organic matter and reduction of pollutants during composting	176	183	187	125	65	61
6	Creation of novel and efficient animal vaccines	6	17	9	12	8	0
7	Preparation of feed by pre-digestion fermentation bioprocessing	0	23	21	15	9	5
8	Genomic mating breeding technology for livestock and poultry	85	106	142	138	205	180
9	Key technologies for unmanned farms	127	171	144	167	219	172
10	Bio-refinery of wood waste	9	10	6	2	7	12
11	Ecological breeding technology of aquatic animals	5	1	3	5	25	13

of important agronomic traits and created new elite germplasms in a variety of crops. However, since the original core patents of genome editing nucleases are monopolized by a few foreign countries such as the USA (Cas9 and Cas12a, etc.), the industrial application of genome editing products of crops in China faces the potential risk of being controlled by others. Therefore, breaking the patent monopoly of genome editing technology, discovering new genome editing nucleases with China's independent intellectual property rights, and developing corresponding genome editing systems such as gene knockout, base editing, DNA fragment insertion and replacement in crops are of great significance for ensuring the safety of genome editing technology industry in China.

## (2) Crop green super-high-yield cultivation technolgy

Crop green super-high-yield cultivation refers to the cultivation engineering technology pattern that steadily and reliably achieves the ecological and environmental friendliness of fields, the efficient utilization of resources, the high quality of crops, and the yield per unit area that can be reproduced or break through the top level in the region. With high demand for agricultural products in China and few resources such as arable land per capita, the search for higher crop yields on limited arable land is undoubtedly a basic orientation that fits the national situation. Over the long term, China's field crops in the cultivation of super-high yield has been explored in various aspects, has emerged more high-yield paradigm, creating a number of super-high yield records. However, it has focused on the breakthrough of single yield in the past, and insufficient attention has been paid to the carbon sequestration and emission reduction of the full amount of straw returned to the field, and the green and efficient use of fertilizers and water chemicals in practice. Research on the super-high yield, high-quality and high-efficiency synergistic formation of the regulation and mechanism is also lacking, the formation of super-high-yield cultivation technology is poorly stabilized, and it is difficult to replicate and popularize it in large-area operation. Therefore, in the coming times, China is in more urgent need to improve the self-sufficiency rate of agricultural products, for the above problems, in-depth study of field crops green super-yield regulation and technology, to accelerate the improvement of the self-sufficiency rate of agricultural products is particularly important. The key scientific problems include: 1) the formation pattern of green ultra-high yield of field crops and the mechanism and regulation pathway of expanding reservoirs and strong sources of steady flow; ② green super-high yield planting methods and new patterns of cultivation; ③ soil carbon sequestration and fertilization techniques for full quantity and pollution-free return of super-high yielding straw to the field; 4 mechanism and technology of green and efficient utilization of fertilizer and water chemicals under super-high yield condition; and (5) integration and replicable demonstration of regionalized green super-high yield mechanical cultivation technology. With independent innovation in crop cultivation and cross-fertilization with physiology, environmental

ecology, informatics, mechanical engineering and other cutting-edge sciences, new theories and technologies of green and superhigh-yield cultivation of crops with Chinese characteristics will be created to achieve synergistic breakthroughs in yield, quality, productivity and to increase more than 30% of production efficiency of field crops, and the comprehensive production capacity of crops, effectively promoting the modernization of China's crop production.

#### (3) Development and utilization of high-quality germplasm resources for horticultural crops

Horticultural germplasm resources refer to the collective name of horticultural plants carrying different germplasm, including cultivated varieties (variations, cultivars, and types) of various horticultural crops, wild species, wild and semi-wild relatives, as well as artificially created varieties or lines, or genetic materials. Horticultural germplasm resources are the material foundation for the exploration and innovation of high-quality germplasm, genetic breeding of horticultural crops, and the development of the industry. China, as the origin of numerous horticultural crops, possesses abundant horticultural germplasm resources. However, there is still insufficient work in utilizing and developing these resources, especially in terms of high-quality resources. The core technological needs include: ① collection, evaluation, and utilization of high-quality germplasm resources of horticultural crops, establishing a high-quality horticultural crop germplasm resource bank; ② innovation and application of multi-trait, high-throughput, and precision phenotypic identification technologies for horticultural crops; ③ exploration and application of high-quality relevant genes in wild germplasm resources, functional analysis of important genes for quality, and regulation networks; and ④ creation of high-quality germplasm resources in horticultural crops through sexual hybridization, physical and chemical mutagenesis, genetic engineering, and other methods. Breaking through these related technologies will greatly promote the breeding of high-quality horticultural crops in China.

#### (4) Molecular design of green pesticides based on structural biology

The conventional approach to pesticide development involves generating a vast array of lead compounds from diverse chemical precursors, followed by direct organism testing and iterative design-synthesis-test-analysis cycles to optimize their efficacy, ultimately leading to the discovery of novel pesticides. According to statistics, the successful development of a new pesticide necessitates the synthesis of 140 000 compounds over a span exceeding 10 years, requiring an investment of nearly 300 million USD. Unfortunately, research and development costs and cycles are continuing to rise. To address these challenges, the molecular design of pesticides based on three-dimensional structural information of molecular targets has emerged as the prevailing approach in novel green pesticide development. Molecular design of green pesticides based on structural biology involves two main technical directions. Firstly, acquiring the three-dimensional structural information of the target and understanding the interaction between the target molecule and the active small molecule are essential prerequisites for molecular design. By analyzing the structural characteristics of the target active sites, elucidating the key motifs involved in small molecule binding, and discerning the structural disparities of the targets among different species, it can provide a foundation for efficient and specific design of green pesticides. In recent years, with the rapid advancement of structural biology technologies such as cryoelectron microscopy, an increasing number of pesticide molecular targets have been obtained, such as chitin synthase and nicotinic receptor. However, the available pesticide molecular target structures are still scarce, and further research is warranted. Second, the utilization of structure-based molecular design techniques is crucial in expediting the research and development cycle. The process of molecular design necessitates a comprehensive consideration of various key aspects, including virtual screening of initial compounds, optimization from initial to lead compounds, druggability analysis of compounds, prediction of physicochemical and toxicological properties, selection of candidate compounds, and other crucial steps. Establishing a structurebased molecular design system and platform for developing green pesticides is essential to facilitate these steps.

# (5) Synergistic technology for efficient conversion of organic matter and reduction of pollutants during composting

The term "efficient conversion of organic matter and reduction of pollutants during composting" describes the process of converting organic matter into stable humus. This is achieved through the use of functional microorganisms, which work under specific conditions of moisture, material ratio, and oxygen concentration. The process is manually controlled to promote humification and reduce emissions of greenhouse gases, odors, and other pollutants caused by the mineralization of composting.

## The core technology include:

- 1) Screen microorganisms that can both degrade lignocellulose and synthesize humus. Research and develop composite microbial agents that use biochar as a carrier. Achieve synergistic and directed humification of organic matter while reducing pollutant gas emissions during the composting process.
- 2) Based on the concept of "waste-to-resource utilization", identify the best additive materials to regulate humification and reduce pollutants during various stages of composting, and then construct and optimize the phased material-mediated technology system to realize the targeted regulation of the composting process.
- 3) Explore the microbial mechanisms of pollutant gas emission mediated by exogenous materials, such as physical, chemical, and biological materials. Elucidate the rules of pollutant emission and innovate technologies for pollutant gas target regulation and nutrient sequestration.
- 4) Research and develop ventilation systems, monitoring systems, and remote-control equipment and systems. Innovative intelligent membrane materials including nanoporous carbon scaffold films.

Using relevant technological breakthroughs is key to the safe and resourceful utilization of agricultural organic waste. It can also provide technical support and solutions for the ecological development of agricultural resources.

## (6) Creation of novel and efficient animal vaccines

Animal vaccine a kind of biological products used to protect animals from specific diseases or pathogen infections. They work by introducing inactivated or attenuated pathogens, partial components, or genetic information, which activate the animal's immune system to generate immune protective responses, thereby enhancing the animal's resistance to diseases. In China, the livestock and poultry farming industry continues to grow in scale and density, meanwhile, the population of companion animals is continually increasing. Major impacted animal diseases and zoonotic diseases seriously threaten the stable development of the industry and human health. Efficient and safe vaccine immunization is one of the core measures for disease prevention and control. Compared to Europe and America, China's innovation level is relatively insufficient in the fields of novel animal vaccine original technology platforms, antigen selection and delivery, and adjuvant development. There is significant room for improvement. Current technological directions and development trends in this field mainly include: ① research and development of universal technologies and platform for screening and delivery of novel vaccines; ② precise target selection and design of novel vaccines for animals; 3 development of novel vaccines with safety and effectiveness, including mucosal immune vaccines, subunit vaccines, gene-deleted marker vaccines, multi-epitope vaccines, live carrier vaccines, nucleic acid vaccines, transgenic plant-edible vaccines, and others; @ strategies for DVAL vaccines that can distinguish vaccinated and infected animal along with the differential diagnostic test kits; (5) establishment of a comprehensive system for monitoring animal diseases and evaluating vaccine immunization effectiveness; and (6) development of key technologies for vaccine production processes. Technological progress in these field will provide technical support and solutions for the prevention, control and eradication of major animal diseases in our country.

#### (7) Preparation of feed by pre-digestion fermentation bioprocessing

Feed pre-digestion technology is the inevitable result of the increasing market demand of low and non-resistant feeding and personalized characteristic livestock products. Biological pre-digestion fermentation of feed is the pre-digestion of feed through enzymatic hydrolysis or microbial fermentation technology, which degrades macromolecular nutrients into small molecular substances easily absorbed by animals, reduces toxic and harmful substances and anti-nutritional factors in feed, improves feed palatability, increases crude protein content in feed, improves digestion and absorption efficiency of feed nutrients, and promotes efficient growth of livestock and poultry. The bioprocessing and preparation system of feed pre-digested fermentation mainly includes: ① analyzing the characteristics of pre-digested feed raw materials, judging the potential digestion key points according to the spatial structure and chemical bond types of raw materials, and identifying the key enzymes required; ② screening of high-quality and safe fermentation strains, in-depth study of their enzyme production types and characteristics, mining enzyme

production genes and regulation of efficient expression, including bacteria-enzyme coordination; ③ optimize the fermentation process, regulate the fermentation conditions such as temperature, pH and time, create a good fermentation environment, and give full play to the high catalytic efficiency and potential of the enzyme; and ④ evaluation of application effects, multi-level evaluation of the effectiveness and safety of pre-digested fermentation products through in vivo and in vitro, multi-level livestock and poultry breeding and validation tests. The development of safe, healthy, efficient and environmentally friendly new feed products is of great significance to promote the efficient and sustainable development of livestock and poultry industry and improve the economic benefits of animal husbandry.

#### (8) Genomic mating breeding technology for livestock and poultry

Genomic mating (GM) breeding technology for livestock and poultry refers to the optimized selection and mating of candidate individuals using their genomic information. It enables control of the rate of inbreeding in the population and facilitates longterm and sustainable genetic progress. However, with the development of genomic selection technology, while pursuing extreme genetic progress for specific traits, there is a significant decrease in the genetic diversity of the population, limiting the selection space for other traits and posing risks of inbreeding depression among high-yielding animals due to higher kinship coefficients. As a result, the optimization of mating based on genomic information has gained increasing attention. It involves comprehensive considerations such as controlling inbreeding, maintaining population genetic diversity, and maximizing genetic progress to formulate sustainable breeding plans. Currently, the U.S. Dairy Cattle Breeding Council has implemented the application of genomic mating in dairy cattle populations, and compared to random mating, genomic mating technology has been shown to increase the value of dam-offspring pairs by \$78 per head. Achieving optimized selection and mating in breeding animals through GM requires consideration of not only the genetic characteristics of the animals themselves but also the genetic relationships and complementarity between mating partners, as well as specific features and traits unique to the breeds. This minimizes the reduction in population genetic heterozygosity, the increase in inbreeding coefficients, and the changes in genetic background gene frequency between selection generations. Therefore, GM is a highly complex problem. Current research mostly focuses on the additive genetic effects of single traits, while future research directions will include multi-trait systems and non-additive genetic effects. In addition to optimizing mating schemes, GM methods can also be applied in the following areas: 1) discovering unknown ancestors or correcting inaccurate pedigrees; 2 estimating the breed composition of hybrid animals or confirming breed purity; ③ avoiding inbreeding and estimating non-additive genetic effects at the genomic level; ④ identifying and monitoring individuals with new defects or economically important traits; and ⑤ predicting the probability of occurrence of high-yielding or low-yielding individuals, among other scenarios or application domains. Therefore, GM is applicable not only for constructing core breeding systems in endangered or critically endangered local species but also for building core breeding systems for high-yielding animals. This will lay the technological foundation for promoting and applying GM in optimized breeding practices.

#### (9) Key technologies for unmanned farms

Currently, in China, the rural labor force is experiencing a growing trend of "aging, feminization, and sidelineization", while the issue of "who will cultivate the land" has become a common challenge for both our country's agriculture and global agriculture. Practical experience has shown that adopting technologies such as intelligent agricultural machinery and smart decision-making management in unmanned farming can significantly enhance labor productivity, land yield, and resource utilization efficiency. The effective approach to addressing the issue of "who will cultivate the land" is the development of smart agriculture, with unmanned farms being a crucial avenue for achieving this. Key technologies for unmanned farms include:

- 1) Digital sensing technology, including accurate sensing technology of the operating environment, operating objects and operating machinery information of unmanned farms, as well as internet of things (IoT) technology to realize the digitization of the whole link and the whole process.
- 2) Intelligent decision-making technology, which combines technologies such as big data, cloud computing, and artificial intelligence. This involves research in data mining, knowledge discovery, and other techniques to achieve intelligent agricultural production decision-making.

- 3) Precision operations technology, utilizing intelligent agricultural machinery equipment, multi-machine coordination, autonomous driving, and precise operation technologies. This enables unmanned intelligent equipment to perform autonomous and precise operations.
- 4) Smart management technology, including crop growth management, agricultural machinery management, and farm management. This facilitates highly coordinated operations for agricultural production processes like plowing, sowing, managing, and harvesting, making the entire farm production process smart and efficient.

Breakthroughs in core technologies of unmanned farms will provide technical support and solutions for addressing the "who will cultivate the land" issue, accelerating the large-scale application of unmanned farms and promoting the development of smart agriculture.

## (10) Bio-refinery of wood waste

Bio-refinery of wood waste, also known as forest biomass refining (compared with petroleum refining), is a technology that uses waste wood fiber biomass generated during wood harvesting and processing as raw materials through thermochemical and biological treatment methods to produce liquid fuels (such as fuel ethanol, biodiesel, etc.), platform chemicals (such as furfural, furfuryl alcohol, etc.), feed additives, bio-based materials, and other products. This technology includes two routes: bioconversion and thermochemical conversion. Bioconversion is a technology that uses microorganisms or enzymes as catalysts to convert biomass into green products through industrial biotechnology. The main research directions of bioconversion include: the technology of extracting, separating, and utilizing secondary metabolites from forest sources mainly included of active components such as flavonoids, tannins, terpenoids, and phenylpropanoids; development and preparation technology of material precursor monomers included of lactic acid, ethylene glycol, succinic acid, and furan dicarboxylic acid materials; fuel preparation technology mainly focused on the preparation of fuel chemicals such as natural gas, cellulose ethanol, and butanol. Thermochemical conversion is a technology that converts wood fibers through pyrolysis, liquefaction, gasification, and other methods. The main research directions of thermochemical conversion include: pyrolysis-gas-carbon coproduction technology for preparing high-quality gas and advanced carbon material precursors; liquefaction technology for preparing platform chemicals such as furfural and phenols, as well as liquid fuels; the preparation technology of bio-based functional materials using platform chemicals as monomers or cellulose, lignin, etc. as matrices. In recent years, with the innovation of biomass refining technology, China has shortcomings in basic theoretical innovation, key core and forward-looking technology reserves. The main directions for future industry development are: ① with the main goal of diversified utilization of raw materials, efficient transformation systems, and high value products, building a full industry chain technology system from renewable raw materials to end products; (2) research and development of cross coupling technologies for bioconversion and thermochemical conversion, as well as key equipment development and technical system construction.

#### (11) Ecological breeding technology of aquatic animals

Ecological breeding technology of aquatic animals is to reduce manual operations and simulate natural environmental conditions of ovulation to maintain the animal welfare of the parents, reduce the loss of the parents, make the parents reusable, and achieve a breeding technology that "simulate nature with less artificial spawning". The concept is to strive to allow aquatic animals to be fertilized naturally in a simulated ecological environment, with little or no use of hormones to induce ovulation, and to collect sperm and eggs with as little human intervention as possible, so that aquatic animal breeding can return to nature. In recent years, with the rapid development of the breeding industry, the demand for aquatic animal seeds in my country has increased sharply. However, traditional breeding operations are not conducive to the sustainable utilization of parents, causing huge economic losses and are not conducive to animal welfare; in addition, artificial hormones The use not only affects the normal physiological activities of aquatic animals, but also brings the risk of environmental pollution. Therefore, carrying out ecological breeding of aquatic animals is an important strategy for the industry to transform into a green and ecological industry. The core technologies include: ① ecological parent breeding technology; ② simulated natural ecological conditions to induce ovulation and fertilization;



and ③ ecological hatching and fry culturing. Relevant technological breakthroughs will promote the ecological transformation of aquatic animal breeding and promote the high-quality development of ecological fisheries.

## 2.2 Interpretations for three key engineering development fronts

#### 2.2.1 Molecular design of green pesticides based on structural biology

The process of molecular design of green pesticides based on structural biology primarily involves the rational and precise development of environmentally friendly pesticides by utilizing three-dimensional structural information of pesticide molecular targets. The creation of green pesticides constitutes a highly intricate multidisciplinary integrated system engineering endeavor characterized by substantial investment, lengthy duration, and significant risk. According to statistics, the development of a new pesticide using traditional methods requires the synthesis of 140 000 compounds and lasts over 10 years with an investment of nearly 300 million USD. In recent years, research and development costs and cycles have continued to increase. However, by employing structural biology methods to acquire key site information for the target, combined with high-throughput virtual screening and computer-aided drug design, the research and development cycle can be expedited, and costs can be reduced.

The development stage of protein structural biology and computer-aided drug design research both commenced in the 1960s. Initially, the integration of these two technologies to establish drug design based on structural biology was primarily employed in pharmaceutical development, subsequently finding applications in the field of pesticides. The article "Current challenges and trends in the discovery of agrochemicals" states that structure-based design is a growing discipline within crop protection research (Lamberth et al., Science, 2013). Currently, structure-based molecular design has become the dominant approach for developing novel green pesticides, leading to the successful creation of numerous innovative green pesticides. For example, based on the structure of succinate dehydrogenase (SDH), fluorophenylamide and chlorophenylamide, which is efficient for the control of rice sheath blight and potato late blight were designed and developed using the method of pharmacophore-linked fragment virtual screening (PFVS). Additionally, new skeleton structures of herbicides, quinoxalone and methylquinoxalone, were developed using a molecular design strategy targeting the conformational changes of the key residue Gln293 in the active pocket of 4-hydroxyphenylpyruvate dioxygenase (HPPD). Moreover, by employing virtual screening based on the structure of the abscisic acid (ABA) receptor, a new ABA receptor agonist called Opabactin (OP) was identified.

By leveraging the structure of a molecular target, dozens or even hundreds of pesticides can be designed and developed. In the case of insecticides, more than 80% of the world's insecticides utilize four structure available molecular targets, namely nicotinic acetylcholine receptor, acetylcholinesterase, GABA-gated chloride ion channel, and pressure-controlled sodium ion channel. Cryoelectron microscopy technology and artificial intelligence have significantly advanced this field in recent years. Cryo-electron microscopy enables the analysis of previously challenging molecular targets in pesticide research, such as chitinase synthase (the potential target for benzoylurea pesticides) and ryanodine receptor (the target for bisamide pesticides). The application of artificial intelligence technology extends beyond target structure prediction, injecting new vitality into the iteration and innovation of pesticide molecular design methods, thereby further expediting the development cycle.

The core patents related to "molecular design of green pesticides based on structural biology" are mainly produced by countries and institutions shown in Tables 2.2.1 and 2.2.2, respectively. Cooperation networks among major countries and institutions are shown in Figures 2.2.1 and 2.2.2. The USA has the most disclosed core patents, with 54 patents, accounting for 48.65%. Denmark ranks second with 24 patents, accounting for 21.62%. Germany ranks third with 16 patents, accounting for 14.41%. China holds 7 patents, ranking fifth globally and accounting for 6.31%. There is cooperation between the USA, Denmark, Sweden, Germany, and Australia; there is no cooperation among other countries. The institution that produced the most core patent was the Novozymes BioAg Limited from Denmark, with a total of 29 patents, followed by University of California and Bayer Crop Science. The top four institutions in terms of citation ratio are Novozymes BioAg Limited (50.44%), University of California (13.64%), Concentric Ag Corporation (7.58%), and Inocucor Technologies Incorporated (7.58%). The institution with the highest citations per patent

Table 2.2.1 Countries with greatest output of core patents on "molecular design of green pesticides based on structural biology"

No.	Country	Published patents	Percentage of published patents/%	Citations	Percentage of citations/%	Citations per patent
1	USA	54	48.65	1 064	48.87	19.70
2	Denmark	24	21.62	944	43.36	39.33
3	Germany	16	14.41	146	6.71	9.12
4	UK	8	7.21	41	1.88	5.12
5	China	7	6.31	21	0.96	3.00
6	Australia	3	2.70	47	2.16	15.67
7	Belgium	2	1.80	42	1.93	21.00
8	New Zealand	2	1.80	0	0.00	0.00
9	Canada	1	0.90	33	1.52	33.00
10	Sweden	1	0.90	10	0.46	10.00

Table 2.2.2 Institutions with greatest output of core patents on "molecular design of green pesticides based on structural biology"

No.	Institution	Published patents	Percentage of published patents/%	Citations	Percentage of citations/%	Citations per patent
1	Novozymes BioAg Limited	29	26.13	1 098	50.44	37.86
2	University of California	10	9.01	297	13.64	29.70
3	Bayer Crop Science	10	9.01	87	4.00	8.70
4	Badische Anilin-und-Soda-Fabrik (BASF)	9	8.11	113	5.19	12.56
5	E.I. Dupont De Nemours and Company	9	8.11	79	3.63	8.78
6	The Secretary of State for Environment Food and Rural Affairs	8	7.21	41	1.8	5.12
7	University of Durham	8	7.21	41	1.88	5.12
8	AgroSpheres Incorporated	7	6.31	84	3.86	12.00
9	Concentric Ag Corporation	5	4.50	165	7.58	33.00
10	Inocucor Technologies Incorporated	5	4.50	165	7.58	33.00

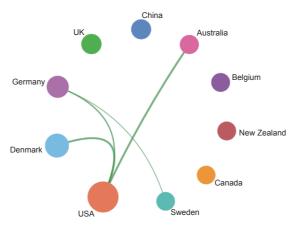


Figure 2.2.1 Cooperation network among major countries in the engineering development front of "molecular design of green pesticides based on structural biology"

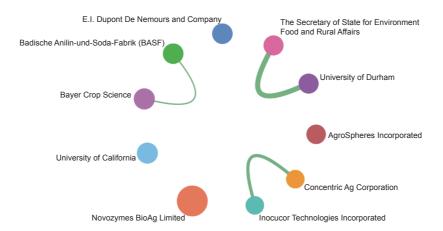


Figure 2.2.2 Cooperation network among major institutions in the engineering development front of "molecular design of green pesticides based on structural biology"

is Novozymes BioAg Limited with 37.86 times. There is cooperation between Bayer Crop Science and Badische Anilin-und-Soda-Fabrik (BASF), the Secretary of State for Environment Food and Rural Affairs and University of Durham, Concentric Ag Corporation, and Inocucor Technologies Incorporated.

Figure 2.2.3 shows the development path of the front of "molecular design of green pesticides based on structural biology". In the next ten years, the engineering frontier of structure-based molecular design of green pesticides is expected to make progress in the following two aspects:

- 1) Technological innovation: With the continuous development of emerging technologies such as artificial intelligence, computer-aided technology, and big data, the field of structure-based green pesticide molecular design is also constantly innovating. For example, artificial intelligence and computer-aided technology can be used to develop new pesticide molecular design methods and new platforms for rational drug design; based on computer-aided design platforms, a small molecule compound library covering a wide chemical space can be constructed and high-throughput screening methods can be developed to design new pesticide molecular skeletons that are easy to modify and derive.
- 2) Environmental protection: Green pesticide molecular design also needs to consider its impact on the environment. For example, new methods for rational drug design based on target resistance prediction can be developed to reduce the risk of resistance and interactive resistance in new pesticide creation from the source. In addition, the environmental safety of pesticide molecules can be evaluated by studying the metabolic behavior of pesticides in the environment, the safety of metabolic products, and factors such as metabolic half-life.

In summary, the field of structure-based green pesticide molecular design is constantly innovating and developing, aiming to achieve safer, more efficient, and more environmentally friendly pesticide production through technological innovation and



Figure 2.2.3 Roadmap of the engineering development front of "molecular design of green pesticides based on structural biology"

environmental protection. This field still faces many challenges and requires continuous exploration and innovation to make greater progress.

## 2.2.2 Preparation of feed by pre-digestion fermentation bioprocessing

Feed pre-digestion technology is the inevitable result of the increasing market demand of low and non-resistant feeding and personalized characteristic livestock products. Biological pre-digestion fermentation of feed is the pre-digestion of feed through enzymatic hydrolysis or microbial fermentation technology, which degrades macromolecular nutrients into small molecular substances easily absorbed by animals, reduces toxic and harmful substances and anti-nutritional factors in feed, improves feed palatability, increases crude protein content in feed, improves digestion and absorption efficiency of feed nutrients, and promotes efficient growth of livestock and poultry. Pre-digested feed contains both protein raw materials (fermented soybean meal, fermented cottonseed protein, baked soybean, hydrolyzed fish protein meal, cottonseed enzymatic hydrolysis protein, wheat hydrolyzed protein) and energy raw materials (baked corn, pre-gelatinized rice), which are important components of feed products. Calculated according to 20% of the output value of feed products, the potential of the output value of pre-digested raw materials is as high as 200 billion yuan.

The development of feed pre-digestion fermentation technology in China can be summarized into three stages. The first stage was saccharification feed and silage in the 1980s. In the second stage, in the 1990s, probiotics were made into bacteriotics and added into the base diet as feed additives until the development of the current stage of microbial fermentation pre-digested feed. In recent years, due to the greatly increased social demand for livestock products, the lack of high-quality protein feed raw materials and the harm caused by long-term use of antibiotics have become increasingly prominent, and feed pre-digestion fermentation technology has been rapidly developed. At present, feed pre-digested fermentation has been widely studied and applied in livestock production. Researchers can improve intestinal health and growth performance of pigs by adding lactic acid bacteria to fermented liquid feed and grape skin fermented feed. In laying hens, adding soybean meal protein fermented feed to basic diet can increase laying rate, reduce feed to egg ratio and ammonia concentration in feces.

The bioprocessing and preparation system of feed pre-digested fermentation mainly includes: ① analyzing the characteristics of pre-digested feed raw materials, judging the potential digestion key points according to the spatial structure and chemical bond types of raw materials, and identifying the key enzymes required; ② screening of high-quality and safe fermentation strains, in-depth study of their enzyme production types and characteristics, mining enzyme production genes and regulation of efficient expression, including bacteria-enzyme coordination; ③ optimize the fermentation process, regulate the fermentation conditions such as temperature, pH and time, create a good fermentation environment, and give full play to the high catalytic efficiency and potential of the enzyme; and ④ evaluation of application effects, multi-level evaluation of the effectiveness and safety of pre-digested fermentation products through in vivo and in vitro, multi-level livestock and poultry breeding and validation tests. The development of safe, healthy, efficient and environmentally friendly new feed products is of great significance to promote the efficient and sustainable development of livestock and poultry industry and improve the economic benefits of animal husbandry.

The major countries and institutions of the core patents related to "preparation of feed by pre-digestion fermentation bioprocessing" are shown in Tables 2.2.3 and 2.2.4, respectively. The top three countries in terms of core patent disclosure are China (64 patents, accounting for 87.67%), the USA (6 patents, accounting for 8.22%), and Republic of Korea (2 patents, accounting for 2.74%). There is no cooperation between countries in this direction. The institutions with the largest number of core patents is Locus IP Company (4 patents). Qingdao Agricultural University's core patent output ranked second (2 patents); and the other institutions have 1 core patent. The top four institutions in terms of citations were Locus IP Company (10.53%), E.I. Dupont De Nemours and Company (6.58%), Freshwater Fisheries Research Center of Chinese Academy of Fishery Sciences (6.58%), and Nanjing Institute of Fishery Sciences (6.58%). The institutions with the highest citations per patent were E.I. Dupont De Nemours and Company, Freshwater Fisheries Research Center of Chinese Academy of Fishery Sciences, and Nanjing Research Institute of Fishery, all with 5 citations. Nanjing Research Institute of Fishery and Freshwater Fisheries Research Center of Chinese Academy of Fishery Sciences have cooperative relations, but there is no cooperative relationship between other institutions (Figure 2.2.4).

Table 2.2.3 Countries with greatest output of core patents on "preparation of feed by pre-digestion fermentation bioprocessing"

No.	Country	Published patents	Percentage of published patents/%	Citations	Percentage of citations/%	Citations per patent
1	China	64	87.67	62	81.58	0.97
2	USA	6	8.22	13	17.11	2.17
3	Republic of Korea	2	2.74	0	0.00	0.00
4	Slovakia	1	1.37	1	1.32	1.00

Table 2.2.4 Institutions with greatest output of core patents on "preparation of feed by pre-digestion fermentation bioprocessing"

No.	Institution	Published patents	Percentage of published patents/%	Citations	Percentage of citations/%	Citations per patent
1	Locus IP Company	4	5.48	8	10.53	2.00
2	Qingdao Agricultural University	2	2.74	1	1.32	0.50
3	E.I. Dupont De Nemours and Company	1	1.37	5	6.58	5.00
4	Freshwater Fisheries Research Center, CAFS	1	1.37	5	6.58	5.00
5	Nanjing Research Institute of Fishery	1	1.37	5	6.58	5.00
6	Hangzhou Genglan Biotechnology Company Limited	1	1.37	3	3.95	3.00
7	Institute of Dryland Farming Hebei Academy of Agricultural and Forestry Sciences	1	1.37	3	3.95	3.00
8	Kaifeng Jar Jun Biotechnology Company Limited	1	1.37	3	3.95	3.00
9	Shenzhen Hemin Biotechnology Company Limited	1	1.37	3	3.95	3.00
10	Hubei Xipu Biological Technology Co., Ltd.	1	1.37	2	2.63	2.00

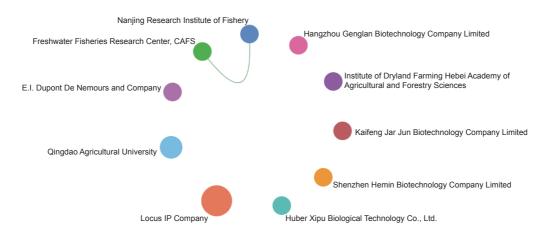


Figure 2.2.4 Cooperation network among major institutions in the engineering development front of "preparation of feed by pre-digestion fermentation bioprocessing"

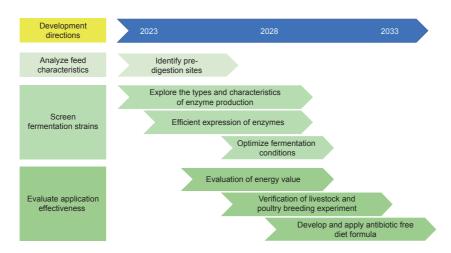


Figure 2.2.5 Roadmap of the engineering development front of "preparation of feed by pre-digestion fermentation bioprocessing"

Figure 2.2.5 shows the roadmap of the engineering development front of "preparation of feed by pre-digestion fermentation bioprocessing".

#### 2.2.3 Ecological breeding technology of aquatic animals

Aquatic animals provide important high-quality protein and high-quality fat for the people. Breeding work on fish, shellfish, shrimps and crabs carried out in recent years has not only greatly increased production, but also improved the quality of cultured products. As the people's demand for a beautiful ecological environment grows, there is an urgent need for aquatic animal breeding to transform from traditional methods to ecological methods. For example, grass carp, which has the largest aquaculture output in my country, requires hormone induction and artificial insemination during the reproduction process. During this process, it is possible to damage the internal organs of the broodstock, and it is also easy to cause skin infection, resulting in the death of a large number of broodstock after birth, which is not conducive to the sustainable utilization of the broodstock, brings huge economic losses, and is not advantageous to animal welfare. In addition, the use of artificial hormones not only affects the normal physiological activities of fish, but also brings the risk of environmental pollution. The purpose of ecological breeding of aquatic animals (Ecological Breeding Technology of Aquatic Animals) is to simulate the natural spawning by reducing artificial operations, maintain the animal welfare of the parents, reduce the loss of the parents, and make the parents reusable, so as to "simulate nature with less artificial spawning". Compared with traditional breeding techniques, ecological breeding started later. Various ecological breeding programs currently being tried have made some progress and can effectively protect high-quality parent resources. The goal of ecological breeding is to strive to allow aquatic animals to be fertilized naturally in a simulated ecological environment by simulating natural ecological breeding conditions, using less or no hormones to induce ovulation, and using less human intervention to collect sperm and eggs, so that aquatic animal breeding can return to nature.

The advantages of ecological breeding of aquatic animals are mainly reflected in following aspects:

- 1) Low damage to parents. Compared with traditional artificial breeding, ecological breeding of aquatic animals only requires building the native environment of the parents, using less or no artificial hormones, and trying to avoid artificial intervention in insemination, which can minimize the loss of parents.
- 2) Environmentally friendly. Ecological animal breeding technology strictly controls the dosage of hormones during the breeding process to avoid burdening the environment.

- 3) The cost is lower. It improves the survival rate of parents after induced ovulation and preserves large-scale parent resources for subsequent breeding. The animal ecological breeding technology only requires a small amount of manual operations, reducing costs.
- 4) Animal welfare. Traditional breeding technology involves a lot of manual operations, while ecological breeding technology is beneficial to ensuring fish welfare through ecological development.

In summary, ecological breeding of aquatic animals is particularly suitable for breeding species with precious parent resources. At the same time, it is also an important technical means that meets the needs of promoting green development and building ecological civilization. It is an aquatic animal breeding strategy with great potential.

Ecological breeding of aquatic animals is a new technology and research hotspot that has emerged with the rise of ecological and environmental protection concepts. It has been carried out in some aquatic animals and has made important progress. However, due to the short development time, it is still in its infancy. Since 2020, nearly 52 results have been published in the field of ecological breeding of aquatic animals. Most of these studies focus on some species that can ovulate and fertilize autonomously under artificial breeding conditions. However, there are few reports on species that require artificial induction, especially fish. The reasons for the lag include: most breeding researchers and operators in breeding farms have adapted to high-intensity, high-yield seed production operations. In order to pursue short-term interests ignore ecological benefits; the understanding of the concept of ecological breeding of aquatic animals is relatively weak, and corresponding systematic research cannot be carried out; there are many aquaculture species, and it is necessary to carry out relevant research on each species and establish an ecological breeding technology system Long-term research, therefore ecological breeding research started late.

In order to overcome difficulties and promote the development of ecological breeding technology for aquatic animals, there is an urgent need to:

- 1) Carry out research on ecological breeding technology, break through technical bottlenecks, clarify key factors and parameters of ecological breeding conditions for key breeding species, and ultimately form a low-cost, standardized, and easy-to-use technology as well as ecological breeding technology process for operation.
- 2) Build an ecological breeding technology system. On the basis of establishing the ecological breeding technology process, we should further carry out small and pilot tests, conduct large-scale experiments according to different species, regions, and breeding models, and obtain systematic ecological breeding model.
- 3) We need to carry out demonstration and promotion of ecological breeding technology in major breeding bases and nurseries, establish high-quality ecological breeding nurseries, and provide standardized examples for the implementation of ecological breeding technology for aquatic animals. In addition, since breeding work involves the consumption of parents, offspring, labor, etc., it requires a large amount of capital investment. Long-term and stable cooperation and investment between research institutions and breeding companies should be promoted.

Table 2.2.5 Countries with greatest output of core patents on "ecological breeding technology of aquatic animals"

No.	Country	Published patents	Percentage of published patents/%	Citations	Percentage of citations/%	Citations per patent
1	China	52	100	25	100	0.48

Table 2.2.6 Institutions with greatest output of core patents on "ecological breeding technology of aquatic animals"

No.	Institution	Published patents	Percentage of published patents/%	Citations	Percentage of citations/%	Citations per patent
1	Fisheries Research Institute, Anhui Academy of Agricultural Sciences	2	3.85	0	0.00	0.00
2	Shenzhen Xingrisheng Industrial Company Limited	2	3.85	0	0.00	0.00
3	Chongqing University of Arts and Sciences	1	1.92	4	16.00	4.00
4	Jiangsu Yuehai Feeds Company Limited	1	1.92	4	16.00	4.00
5	Zhejiang Mariculture Research Institute	1	1.92	4	16.00	4.00
6	South China Agricultural University	1	1.92	3	12.00	3.00
7	Southwest University	1	1.92	2	8.00	2.00
8	Tianjin Aquatic Product Research Institute	1	1.92	2	8.00	2.00
9	Mianyang Anzhou District Xinmin Agricultural Technology Company Limited	1	1.92	1	4.00	1.00
10	Qingdao Ruizi Treasure Development Company Limited	1	1.92	1	4.00	1.00

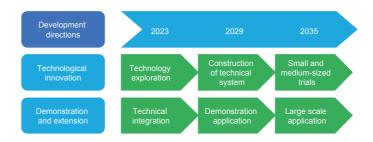


Figure 2.2.6 Roadmap of the engineering development front of "ecological breeding technology of aquatic animals"

The main countries and institutions of the core patents related to "ecological breeding technology for aquatic animals" are shown in Tables 2.2.5 and 2.2.6, respectively. There is no cooperation among major institutions. Figure 2.2.6 shows the development path of the "ecological breeding technology for aquatic animals".

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