

VII. Agriculture

1 Engineering research fronts

1.1 Trends in top 10 engineering research fronts

The top 10 engineering research fronts in the agriculture field can be classified into three categories: (1) In-depth established research fronts. These comprise “mechanisms of plant immunity regulation” of plant protection, “influences of climate change on agroecosystems” of resource ecology, “biodiversity and ecosystem services” of resource ecology, “artificially induced spawning” of fisheries science and “global climate change and crop production” of resource ecology. (2) Emerging research fronts. These comprise “genomic selection breeding of crops” of crop science, “mechanisms, prevention, and control of animal epidemics” of animal science, “intelligent biological factories” of agricultural engineering, and “forest monitoring through hyperspectral remote sensing” of forestry science. (3) Ground breaking research front. This comprises “CRISPR/Cas9 genome editing of agricultural organisms” of agricultural engineering.

The numbers of core articles supporting various fronts were unevenly distributed. There were 30 core articles on average for each front, with an average of 40 citations per article (Table 1.1.1). The articles of all fronts were mainly published in 2015–2017 (Table 1.1.2), with no obvious variation patterns across the years. In particular, the number of published articles on artificially induced spawning exhibited a gradual upward trend with time. Data on relevant publications also showed that the highest proportion of highly cited articles in articles on these fronts was less than 30%, and only an extremely small number of articles had patent citations.

The top 10 engineering research fronts are briefly described below.

(1) CRISPR/Cas9 genome editing of agricultural organisms

This is a ground breaking research front. Genome editing techniques involve the specific cutting of DNA at target sites by endonucleases to generate DNA double-strand breaks (DSBs), which induces DNA repair and enables the achievement of directional genome editing. Among the various genome editing techniques, CRISPR/Cas9 editing is an accurate, effective and convenient technique. CRISPR is a family of

Table 1.1.1 Top 10 engineering research fronts in agriculture

No.	Engineering research front	Core papers	Citations	Citations per paper	Mean year
1	CRISPR/Cas9 genome editing of agricultural organisms	42	3261	77.64	2015.6
2	Mechanisms, prevention, and control of animal epidemics	11	153	13.91	2017.3
3	Genomic selection breeding of crops	6	174	29.00	2017.3
4	Intelligent biological factories	26	547	21.04	2015.8
5	Mechanisms of plant immunity regulation	43	1265	29.42	2015.8
6	Influences of climate change on agroecosystems	11	795	72.27	2016.1
7	Biodiversity and ecosystem services	35	1356	38.74	2015.9
8	Artificially induced spawning	44	704	16.00	2016.5
9	Global climate change and crop production	85	2298	27.04	2015.5
10	Forest monitoring through hyperspectral remote sensing	15	937	62.47	2015.5

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

No.	Engineering research front	2013	2014	2015	2016	2017	2018
1	CRISPR/Cas9 genome editing of agricultural organisms	1	5	14	12	8	2
2	Mechanisms, prevention, and control of animal epidemics	0	0	0	2	4	5
3	Genomic selection breeding of crops	0	0	0	0	4	2
4	Intelligent biological factories	3	4	3	5	3	4
5	Mechanisms of plant immunity regulation	7	5	8	1	12	10
6	Influences of climate change on agroecosystems	1	1	2	2	2	3
7	Biodiversity and ecosystem services	6	4	2	3	14	6
8	Artificially induced spawning	1	2	6	10	14	11
9	Global climate change and crop production	10	11	22	18	19	5
10	Forest monitoring through hyperspectral remote sensing	1	4	3	3	1	3

clustered, regularly interspaced, short palindromic repeat sequences found in nearly all archaeal genomes. It mainly depends on the Cas9 core protein for the RNA-mediated identification of target sequences for DNA cutting, which results in DNA DSBs. During DNA repair, CRISPR provides organisms with a specific immunity protection mechanism to resist invasion by genetic material from plasmids or viruses. Therefore, the CRISPR/Cas9 technique effectively solves the issues of multi-generation crossing and the long duration associated with crossbreeding methods, and enables the directional acceleration of breeding processes.

(2) Mechanisms, prevention, and control of animal epidemics

This is an emerging research front. An animal epidemic occurs when infectious disease or parasitic origin in animals endangers the health and lives of humans and animals, thereby necessitating urgent and harsh mandatory measures for epidemic prevention and control. To date, more than 250 types of infectious animal diseases have been discovered globally. Almost 50% of these diseases affect both humans and animals, which not only cause disease-related deaths of animals, but also produces adverse impacts on animal rearers, consumers, and the broader economy. Scientists typically employ information technologies to study biological data and utilize bioinformatics principles to collect, process, and analyze genomic information on pathogens. Through genome comparisons, the protein structures of pathogens can be confirmed, and novel drugs with enhanced therapeutic effects can be developed based on molecular characteristics that result in pathogenesis. With economic globalization, the

movement of people, goods, and livestock across borders has increased substantially. This has led to the gradual transformation of certain localized outbreaks into globalized diseases and the emergence of alarming public health issues. For instance, bovine spongiform encephalopathy (BSE), first reported in 1986 in the UK, has now spread to more than 20 countries, including Canada, various European countries, and Japan. Given the negative externalities and publicness of animal diseases, various parties have focused their attention on certain major animal diseases and adopted relevant prevention and control strategies. Animal epidemic prevention and control strategies refer to targeted prevention and control schemes or measures such as technological inputs and institutional arrangements to reduce the impact of infectious animal diseases on human society. The prevention and control of animal epidemics is a key influencing factor of the robust development of animal husbandry. Throughout their life, domestic animals are subject to various measures, such as feed, breeding environment and immunization, to prevent the onset of disease and maintain their health; these measures take livestock health, product safety and environmental protection into account and assures the safety of animal products from source.

(3) Genomic selection breeding of crops

This is an emerging research front. Since the 1980s, the rapid development of molecular biology has created possibilities for molecular genetic marker-assisted breeding, i.e., marker-assisted selection (MAS). The advantages of MAS include its high accuracy, absence of sex limitations, rapid enhancement

of favorable allele frequencies or elimination of harmful alleles, and early selection. However, as the mutual validation of quantitative trait loci (QTL) mapping results among various studies is difficult, mapping intervals are excessively large and QTL effects are usually overestimated, the application of MAS in crop breeding is severely limited. To overcome the inadequacies of using a small number of markers for selection, researchers have combined the latest results in functional genomic studies with state-of-the-art single nucleotide polymorphism (SNP) chip technologies. Meuwissen et al. proposed the calculation of the genomic estimated breeding values (GEBVs) of individuals based on high-density markers that cover the whole genome. Compared with estimated breeding values calculated based on genealogical information, GEBVs usually achieve a higher estimation accuracy. Genomic selection breeding (GSB) refers to selection based on the use of GEBVs as the genomic breeding values. The GSB process is as follows. First, a reference population is established, with all individuals of the population having known phenotypes and genotypes. The effect values of each SNP or different chromosome segments are calculated using a suitable statistical model. Then genotyping is performed on each individual of the candidate population, and the GEBVs of the individuals of the candidate population are calculated based on the estimated SNP effect values of the reference population. Finally, individuals are selected based on the ranking of GEBVs. When performance testing of the selected candidates has been completed, these individuals can be included in the reference population for the re-estimation of the SNP effect values, and the entire process is repeated. With the increasing adoption of commercial high-density SNP chips and a decline in sequencing costs, genomic selection has become widely applied in the practice of plant and animal agriculture. The use of high-density SNP chips can accelerate the application of genomic selection in crop breeding.

(4) Intelligent biological factories

This is an emerging research front. At present, the intelligence revolution, which is driven by big data and artificial intelligence, has profoundly changed the connotations and extensions of bioengineering, and achieved transformational results in the fields of crop production, macromolecular design, synthetic biology, and microchemical engineering. Facility agriculture is a modern agriculture method that utilizes engineering technologies and methods for effective

plant production under relatively controllable environmental conditions. In particular, intelligent biological factories, which have emerged as part of the prevailing intelligence revolution, represent the cutting edge in the development of facility agriculture. Intelligent facility agriculture includes facility planting, facility culturing, and facilities producing edible fungi. During the transformation of automated facilities from planting and cultivation to high-level intelligent systems, the collection and monitoring of environmental and biological information (including facility environmental factors and the growing and production processes of organisms) as well as the development of control platforms for intelligent systems, management models of organisms (plants, fungi and livestock), intelligent light sources, intelligent feeding systems, harvesting robots, and Internet of Things (IoT)-based agricultural product quality traceability systems will form the core topics of future research. In addition, synthetic meat products manufactured by intelligent biological factories using animal stem cells will also gradually make their way to the dining tables of consumers in the near future.

(5) Mechanisms of plant immunity regulation

This is an in-depth established research front. Plant disease resistance includes resistance to invasion and infection, resistance to expansion, induced resistance, structural resistance, biochemical resistance, hypersensitive necrotic reaction, and systemic acquired resistance. In natural environments, plants are subject to infection by various types of pathogens. The epidermis serves as the first line of defense against the entry of pathogens, which is a manifestation of non-host resistance. However, when pathogenic organisms successfully invade the internal parts of a plant, the innate immune system of the plant is activated. The immunoregulatory mechanism of the innate immune system consists of four steps: 1) pathogen-associated molecular pattern-triggered immunity (PTI), which involves the detection of molecular patterns on the surfaces of pathogens by pattern recognition receptors; 2) the release of effectors by pathogenic microbes to inhibit PTI and initiate a hypersensitive response (HR) of plant cells toward the pathogens; 3) effector-triggered immunity, which is an immune response triggered by effectors specifically recognized by nucleotide binding leucine-rich repeat proteins; and 4) the disease resistance genes of the plant produces new resistance proteins under natural selection pressure, which induces cell death at the sites of pathogenic

microbial infection. This HR effectively prevents the growth of pathogenic microbes, especially parasites that acquire cell nutrients through their mouthparts, in tissues where HR has occurred. Tissues uninfected by pathogenic microbes do not usually produce a HR.

(6) Influences of climate change on agroecosystems

This is an in-depth established research front. The ecological factors of global climate change that exert key influences on agricultural ecosystems are increased CO₂ concentrations, increased temperatures, and changes in precipitation. An increase in CO₂ concentration promotes the translocation of photosynthetic products of crops to the roots, thereby enhancing carbon fixation by the underground parts of agroecosystems and absorption of water by plant roots, which increases the primary productivity of agroecosystems. Higher soil organic carbon levels provide more degradable substrates for soil microbes, consequently enhancing soil respiration. Global warming leads to a greater input of energy from external environments to agroecosystems, which causes changes in the structures and functions of agroecosystems. By influencing the physiological processes of crops and mutual interactions among species or even altering the genetic characteristics of species, climate change may influence the species compositions, structures, and functions of agroecosystems. Changes in precipitation and rises in global sea levels can also have a direct or indirect impacts on the functioning of agroecosystems. It is evident that global climate change will pose unprecedented severe challenges to mankind.

(7) Biodiversity and ecosystem services

This is an in-depth established research front. Biodiversity refers to the variability and diversity of life forms, such as animals, plants and microbes, existing in terrestrial, marine and other aquatic ecosystems, as well as the complex ecological processes among various constituents of ecosystems. It includes species, ecological environmental, nutritional, life-cycle, and genetic diversities, and contributes greatly to the conservation of natural resources. Ecosystem services are defined as the benefits that humans gain from various ecosystems, and are composed of supporting services, provisioning services, cultural services, regulating services, and the interactions between these services. The biological and physical structures and processes of ecosystems are

manifested in the structural and functional characteristics of ecosystems, and the stability of these ecosystem structures and functions ensures a continuous supply of ecosystem services. The current global decrease in key ecosystem services poses an enormous threat to human society. As biodiversity serves as the provider and service basis of various ecosystem products, the restoration of lost ecosystem services and biodiversity through ecological restoration projects is of great significance to the alleviation of environmental stresses faced by mankind.

(8) Artificially induced spawning

This is an in-depth established research front. The reproductive activities of most fish and shrimp species are seasonal in nature, with only a small number of year-round spawning species. In addition, precision in the timing of reproductive events of various species ensures that the larvae produced are provided with appropriate conditions for survival. Among the various environmental factors, temperature, photoperiod, rainfall and food are the most crucial for regulation of the reproductive cycles of fish and shrimp. In general, the sense organs of fish and shrimp transmit information regarding the changes in these environmental factors to the brain, causing the hypothalamus to secrete gonadotropin-releasing hormone (GnRH) and other neuroendocrine factors. This triggers the secretion of gonadotropic hormones by the pituitary gland, which stimulates the production of gonadal steroid hormones by the gonads, thereby promoting gonadal development and maturation, as well as sperm and egg production. The external environmental factor-activated cyclic reproductive activities of fish and shrimp are regulated by a series of neuroendocrine hormones, with gonadotropins contributing significantly to regulation. In view of these mechanisms, the following approaches are mainly adopted in artificially induced spawning. 1) Environmental stress methods. When significant changes occur in the living environment (e.g., droughts, floods, and extreme hot or cold weather), species continuation and reproductive needs become the first priority of survival in crayfish. Under such circumstances, the reproductive performance of crayfish undergoes certain changes, which are manifested as early reproduction and increased reproduction under environmental stresses. 2) The use of highly effective, low-cost, and side-effect-free novel uterotonic agents, such as high-activity novel uterotonic agents for fish composed

of GnRH analogs (LHRH-A or sGnRHA) and domperidone, a dopamine D2 receptor antagonist.

(9) Global climate change and crop production

This is an in-depth established research front. Since the release of the First Assessment Report of the Intergovernmental Panel on Climate Change in 1990, the investigation of crop production under future climate change scenarios has become extremely important when evaluating the influence of future climate change on agriculture, as it enables the determination of soil production potential and the ability to safeguard food security. The influences of global climate change-induced increases in temperature and atmospheric CO₂ concentrations on crop production must be evaluated on a long timescale. In recent years, researchers have developed a series of models, including ecosystem function, crop growth, net primary productivity, atmospheric, biogeochemical and ecological models, to investigate the relationships between global climate change and crop production. In particular, there has been a considerable amount of crop growth model-based research on the influences of climate change on crop production, growth and development, and the regional climate adaptability of crops. At present, the use of crop growth models for numerical simulation and prediction is the main research method employed in studies on the quantification of the influences of climate change on agricultural production. Such models can also be used to determine the patterns of influence of historical climate change on crops and to analyze the mechanisms by which ecological succession occurs in crop species to achieve adaptation to climate change.

(10) Forest monitoring through hyperspectral remote sensing

This is an emerging research front. Hyperspectral remote sensing is a novel technology involving the acquisition of continuous spectral information on ground objects in extremely narrow and contiguous spectral bands. The combination of imaging and spectroscopy in a single system and an extremely high spectral resolution are unique advantages of hyperspectral remote sensing. Hyperspectral remote sensing utilizes narrow spectral bands to acquire relevant data on ground objects of interest. It enables the acquisition of huge volumes of continuous imaging data in extremely narrow bands in the mid-infrared, visible, near-infrared, and ultraviolet spectral regions, and is mainly used for the following applications. 1) Monitoring of forest fires.

Hyperspectral remote sensing can be used to determine whether surface temperatures are abnormal and whether ground objects are combustible at the surveyed locations. By combining these factors with normal remotely sensed data, the locations of forest fires and consequences of burning can be qualitatively and quantitatively analyzed. 2) Forest pest monitoring. When forests are invaded by pests, the chlorophyll content of plants usually decreases and the intensity of chlorophyll absorption bands consequently weakens, which leads to an increase in the overall visible light reflectance. Information on such changes can be extracted from remotely sensed images to provide a reference for the prevention of pest problems. 3) Monitoring of resource changes in forests. Hyperspectral remote sensing can be used for the monitoring of changes in forest land, ecological assessments of forests, and detailed classification of forest types and tree species.

1.2 Interpretations for three key engineering research fronts

1.2.1 CRISPR/Cas9 gene editing in agricultural organisms

Genome editing techniques have sparked research interest in many countries. In 2012, genome editing was named by *Science* magazine as one of the top 10 scientific advances; in 2014, it was named by *Nature Methods* as one of the top 10 research methods that exerted the greatest influence on biological research in the past decade. Genome editing techniques involve the specific cutting of DNA at target sites by endonucleases to generate DSBs, which induces DNA repair and enables the achievement of the directional genome editing. These techniques effectively solve the problem of multi-generation crossing and the long duration associated with crossbreeding methods and enable the directional acceleration of breeding processes. In addition, because of the artificial increase in mutation efficiency and alteration of the natural evolutionary processes of crops, the environmental safety and food safety risks of genome-edited plants have increased. Genome editing has already progressed to the fourth generation, with zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), meganucleases (MGNs), and CRISPR/Cas9 being the major tools employed in various genome editing techniques. In particular, the CRISPR/Cas9 system is an accurate, effective,

and convenient genome editing tool. Its working principle is as follows. The conserved protospacer adjacent motif (PAM) sequence NGG (N being any nucleotide and G being guanine) in exogenous genes can be recognized by single-guide RNA (sgRNA), which subsequently directs the Cas9 protein to cleave DNA upstream of the PAM. When the cleaved DNA undergoes non-homologous end joining (NHEJ) for the repair of the DSB, small insertion/deletions are created, causing a frameshift mutation which leads to the achievement of gene knockout. The CRISPR/Cas9 system is advantageous in that the sgRNA of CRISPR can recognize the PAM sequence with a guide sequence of no more than 30 nucleotides, and the monomeric Cas9 protein is sufficient to elicit cleavage. Compared to other gene editing tools, the CRISPR/Cas9 system provides a greater ease of operation, higher knockout efficiency, and higher accuracy in gene editing, which greatly reduces the chances of off-target mutations. Therefore, it is currently widely applied in gene editing in key plant and animal species.

The analysis of data on the relevant literature is as follows. From the distribution of papers by country or region (Table 1.2.1), it can be seen that the main contributors of core papers were China and the USA, followed by Germany and Japan. The number of citations was highest in China, followed by the USA and Germany. From the distribution of papers by research institution (Table 1.2.2), it can be seen that the Chinese Academy of Sciences (China) and University of Minnesota (USA) were ranked at the top with seven core articles each, and the number of citations was highest for papers published by the Chinese Academy of Sciences. The network diagram of inter-country/regional collaborations (Figure 1.2.1) shows that inter-country collaborations were common, with China and the USA sharing the closest collaborative relationship; a significant collaborative relationship also existed between the USA and the UK. From the network diagram of collaborations among the major contributing institutions (Figure 1.2.2), it can be seen that a close collaborative relationship existed between the Chinese Academy of Sciences and the University of Chinese Academy of Sciences; certain collaborative relationships also existed among other institutions. The main contributors of core papers citations were China and the USA, with the proportions of core paper citations being substantially higher than that of other countries or regions (Table 1.2.3). From the list of the major core paper citation-contributing institutions (Table 1.2.4), it can be seen that six out of the

top 10 core paper citation-contributing institutions were Chinese institutions. In addition, research institutions located in China (Chinese Academy of Sciences, Chinese Academy of Agricultural Sciences, Huazhong Agricultural University, University of Chinese Academy of Sciences, and China Agricultural University) occupied the top five spots on the list. The average core paper citation year of these institutions was 2017, which was generally later than that of other institutions. This is indicative of the strong developmental momentum of research in this field.

An in-depth analysis of the supporting data revealed that more than 40 relevant research articles had a citation frequency of >200, with most of these articles being reviews. In particular, the article entitled *Development and Applications of CRISPR-Cas9 for Genome Engineering* published in *Cell* in 2014 had a citation frequency of >1700, which provides adequate proof of the leading position of the CRISPR/Cas9 system in the field of genome editing. At present, the CRISPR/Cas9 technique has been widely applied in model organisms such as *Arabidopsis*, yeast, mouse, human, and *Drosophila*, and has even enabled the achievement of fixed-location genome editing in economic animals such as cattle, pigs, and sheep, as well as major crops such as wheat, sorghum, rice, and corn. Certain institutions, including the Chinese Academy of Sciences, Chinese Academy of Agricultural Sciences, and Huazhong Agricultural University, have made tremendous progress in the genetic improvement of agricultural crops, while other institutions, such as the China Agricultural University, hold leading positions in research related to gene editing in economic animals.

1.2.2 Mechanisms, prevention, and control of animal epidemics

Common animal epidemics include foot-and-mouth disease, African swine fever (ASF), contagious bovine pleuropneumonia (lung plague), BSE (mad cow disease), bluetongue disease, and avian influenza (highly pathogenic avian influenza). To date, more than 250 types of infectious animal diseases have been discovered. Almost 50% of these diseases affect both humans and animals, which not only results in disease-related deaths of animals, but also have adverse impacts on animal rearers, consumers, and the broader economy. Based on the overall epidemic situation, infectious diseases can be classified as contagious or non-contagious according

Table 1.2.1 Countries or regions with the greatest output of core papers on “CRISPR/Cas9 genome editing of agricultural organisms”

No.	Country/Region	Core papers	Percentage of core papers	Citations	Percentage of citations	Citations per paper
1	China	17	40.48%	1437	44.07%	84.53
2	USA	17	40.48%	1399	42.90%	82.29
3	Japan	5	11.90%	156	4.78%	31.20
4	Germany	5	11.90%	455	13.95%	91.00
5	South Korea	3	7.14%	171	5.24%	57.00
6	Italy	2	4.76%	99	3.04%	49.50
7	UK	2	4.76%	219	6.72%	109.50
8	Sweden	1	2.38%	47	1.44%	47.00
9	Mexico	1	2.38%	17	0.52%	17.00
10	Philippines	1	2.38%	17	0.52%	17.00

Table 1.2.2 Institutions with the greatest output of core papers on “CRISPR/Cas9 genome editing of agricultural organisms”

No.	Institution	Core papers	Percentage of core papers	Citations	Percentage of citations	Citations per paper
1	Univ Minnesota	7	16.67%	486	14.90%	69.43
2	Chinese Acad Sci	7	16.67%	688	21.10%	98.29
3	Karlsruhe Inst Technol	4	9.52%	395	12.11%	98.75
4	Yokohama City Univ	4	9.52%	116	3.56%	29.00
5	Chinese Acad Agr Sci	4	9.52%	176	5.40%	44.00
6	Seoul Natl Univ	3	7.14%	171	5.24%	57.00
7	Natl Agr & Food Res Org	3	7.14%	77	2.36%	25.67
8	Univ Chinese Acad Sci	3	7.14%	218	6.69%	72.67
9	Inst for Basic Sci Korea	2	4.76%	111	3.40%	55.50
10	Univ Elect Sci & Technol China	2	4.76%	195	5.98%	97.50



Figure 1.2.1 Collaboration network among major countries or regions in the engineering research front of “CRISPR/Cas9 genome editing of agricultural organisms”

to the route of transmission. When the timelines of a single epidemic are examined, it can be seen that major infectious diseases are typical unexpected events which consist of the life cycle stages of onset, development, evolution, and decline. On this basis, the life cycle of a major infectious disease can be divided into the latent, escalation, outbreak, and termination periods. After a long period of latency, a major infectious disease infects animals, which causes the emergence of symptoms. This is followed by an escalation of the epidemic and a massive outbreak within a short period of time. To control an epidemic, measures such as culling, and disinfection are usually adopted. In the absence of new cases, an infectious disease will enter the subsequent stages of decline and termination. The transmission of an infectious

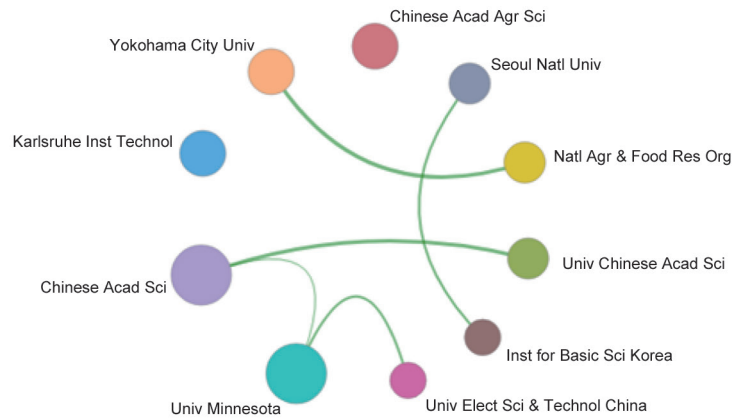


Figure 1.2.2 Collaboration network among major institutions in the engineering research front of “CRISPR/Cas9 genome editing of agricultural organisms”

Table 1.2.3 Countries or regions with the greatest output of citing papers on “CRISPR/Cas9 genome editing of agricultural organisms”

No.	Country/Region	Citing papers	Percentage of citing papers	Mean year
1	China	419	30.17%	2017.2
2	USA	388	27.93%	2016.9
3	Germany	104	7.49%	2016.7
4	UK	93	6.70%	2017.1
5	Japan	78	5.62%	2017.0
6	India	70	5.04%	2017.2
7	Australia	68	4.90%	2017.4
8	France	58	4.18%	2017.2
9	Italy	43	3.10%	2017.0
10	Netherlands	34	2.45%	2017.6

Table 1.2.4 Institutions with the greatest output of citing papers on “CRISPR/Cas9 genome editing of agricultural organisms”

No.	Institution	Citing papers	Percentage of citing papers	Mean year
1	Chinese Acad Sci	100	23.15%	2017.1
2	Chinese Acad Agr Sci	65	15.05%	2017.3
3	Huazhong Agr Univ	52	12.04%	2017.3
4	Univ Chinese Acad Sci	43	9.95%	2017.3
5	China Agr Univ	34	7.87%	2017.3
6	Univ Minnesota	30	6.94%	2016.4
7	Karlsruhe Inst Technol	24	5.56%	2016.3
8	Iowa State Univ	23	5.32%	2016.8
9	Univ Calif Davis	21	4.86%	2017.0
10	South China Agr Univ	20	4.63%	2016.9

disease requires three elements, namely infection sources, transmission routes and susceptible populations. Infection sources refer to humans, animals or plants infected by the pathogens of the infectious disease. Besides surviving and reproducing within the bodies of infection sources, pathogens can also spread to the external environment through the movement of infection sources. Transmission routes are the processes by which pathogens infect humans or animals after being expelled from the infection sources. Both contagious and non-contagious infectious diseases can only infect animals by transmission through certain types of media. ASF is a highly contagious hemorrhagic viral disease caused by the ASF virus and has a mortality rate of up to 100%. Since the first report of the disease in 1921, it has primarily circulated within sub-Saharan Africa until the occurrence of an outbreak in Georgia in 2007. Subsequently, the epidemic spread to the entire Caucasus region, including Russia. In 2014, ASF spread to most countries in eastern Europe and showed initial signs of escalation. In China, the first ASF outbreak was reported in August 2018 and was followed by a rapid spread of the disease within the country. Consequently, the World Organization for Animal Health (OIE) listed ASF as a reportable animal disease. At present, there are no commercial vaccines available for ASF. BSE is a chronic death-causing neurodegenerative disease of cattle mainly characterized by the occurrence of spongiform lesions in the gray matter. The primary etiological agent of BSE is the prion, which is an infectious protein with the ability to self-replicate. Avian influenza, commonly known as bird flu, is an acute infectious disease. Although this virus mainly affects birds, it can also infect humans and has been listed as a Category A infectious disease by the OIE. The prevention and control of animal epidemics is fundamentally dependent on the research, development, and mass production of

vaccines. As the protective effects of subunit, nucleic acid and viral vector vaccines are generally weak, the primary goal of researchers worldwide is the development of gene-deleted vaccines, which can provide complete protection.

From the distribution of papers by country or region, it can be seen that the main contributors of core papers were Brazil, the USA, Germany, and China (Table 1.2.5). The distribution of papers by research institution shows that the number of core papers and number of citations were the highest for Iowa State University (USA) and Boehringer Ingelheim Vetmedica, Inc. (Germany) (Table 1.2.6). The network diagram of collaborations among the major countries or regions indicates a close collaborative relationship between the USA and Brazil, while China had relatively fewer collaborations with other countries. From the network of collaborations among the various research institutions, it can be seen that the collaborations were mostly concentrated in two clusters: collaborations between US and Brazilian institutions, and collaborations between German and Polish institutions (Figures 1.2.3 and 1.2.4). Core paper citations were mainly contributed by China, Germany, and Brazil (Table 1.2.7). The average citation year of core paper was also relatively late, which is indicative of the strong developmental momentum of research in this field (Table 1.2.8).

1.2.3 Genomic selection breeding of crops

The GSB of crops refers to the construction of genomic technical tools for crop breeding through the combination of the latest results in functional genomic studies with state-of-the-art SNP chip technologies developed by international researchers. Through such methods, crop breeders can

Table 1.2.5 Countries or regions with the greatest output of core papers on “mechanisms, prevention, and control of animal epidemics”

No.	Country/Region	Core papers	Percentage of core papers	Citations	Percentage of citations	Citations per paper
1	Brazil	3	27.27%	18	11.76%	6.00
2	USA	3	27.27%	45	29.41%	15.00
3	Germany	3	27.27%	52	33.99%	17.33
4	China	2	18.18%	16	10.46%	8.00
5	Spain	1	9.09%	9	5.88%	9.00
6	Austria	1	9.09%	24	15.69%	24.00
7	Poland	1	9.09%	3	1.96%	3.00

Table 1.2.6 Institutions with the greatest output of core papers on “mechanisms, prevention, and control of animal epidemics”

No.	Institution	Core papers	Percentage of core papers	Citations	Percentage of citations	Citations per paper
1	Iowa State Univ	3	27.27%	45	29.41%	15.00
2	Boehringer Ingelheim Vetmed Inc	2	18.18%	40	26.14%	20.00
3	Sao Paulo State Univ Unesp	2	18.18%	11	7.19%	5.50
4	Vet Resources Inc	2	18.18%	11	7.19%	5.50
5	Univ Vet Med	2	18.18%	37	24.18%	18.50
6	Generalitat Catalunya	1	9.09%	9	5.88%	9.00
7	Inst Agrifood Res Tech	1	9.09%	9	5.88%	9.00
8	Univ Alabama Birmingham	1	9.09%	9	5.88%	9.00
9	Univ Autonoma Barcelona	1	9.09%	9	5.88%	9.00
10	Traunkreis Vet Clin	1	9.09%	24	15.69%	24.00

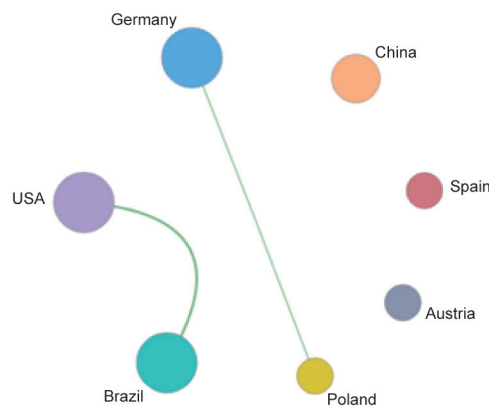


Figure 1.2.3 Collaboration network among major countries or regions in the engineering research front of “mechanisms, prevention, and control of animal epidemics”

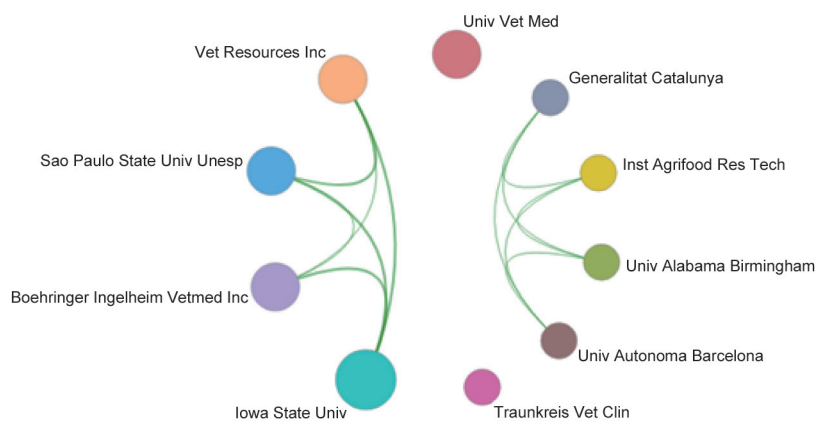


Figure 1.2.4 Collaboration network among major institutions in the engineering research front of “mechanisms, prevention, and control of animal epidemics”

Table 1.2.7 Countries or regions with the greatest output of citing papers on “mechanisms, prevention, and control of animal epidemics”

No.	Country/Region	Citing core papers	Percentage of citing papers	Mean year
1	China	14	25.93%	2018.0
2	Germany	12	22.22%	2017.3
3	Brazil	10	18.52%	2017.9
4	USA	5	9.26%	2017.6
5	Spain	4	7.41%	2017.8
6	Italy	2	3.70%	2017.5
7	Austria	2	3.70%	2017.0
8	Netherlands	2	3.70%	2017.0
9	Serbia	1	1.85%	2017.0
10	Taiwan of China	1	1.85%	2017.0

Table 1.2.8 Institutions with the greatest output of citing papers on “mechanisms, prevention, and control of animal epidemics”

No.	Institution	Citing core papers	Percentage of citing papers	Mean year
1	Univ Vet Med	9	22.50%	2017.2
2	South China Agr Univ	6	15.00%	2017.7
3	Univ Vet Med Hannover	4	10.00%	2018.0
4	Sao Paulo State Univ Unesp	4	10.00%	2018.0
5	Univ Med Ctr Hamburg Eppendorf	3	7.50%	2017.3
6	Univ Autonoma Barcelona	3	7.50%	2017.7
7	Iowa State Univ	3	7.50%	2017.7
8	Heinrich Pette Inst	2	5.00%	2016.5
9	German Ctr Infect Res	2	5.00%	2018.5
10	Generalitat Catalunya	2	5.00%	2017.5

perform laboratory testing of the seeds or seedlings of crops, accurately identify and screen individuals with good genes, and predict trait expression in crops in the field. This enables the enhancement of the purposefulness and targetedness of breeding processes, and the accurate selection and consolidation of multiple suitable gene loci. Strains with the greatest potential in trait expression can then be selected for the breeding of new crop cultivars, thereby achieving the scientific control of the breeding process and providing a means for realizing the ultimate goal of designed breeding. Research has shown that chips for the genomic breeding of crops can be useful in germplasm diversity analysis, gene identification, gene mapping, the selection of genotypes as breeding materials and genetic fingerprinting of cultivars. In actual practice, a specific training group of crops can be

designed for the collection of genotype data, phenotype data and related environmental factors (e.g., geographical differences, processing methods during testing and seasons), and specific model construction algorithms can be used to construct the training model, which calculates the breeding values of individuals or the contributions of each marker to the traits of the crop, as well as the predicted phenotype values of the test group. Model accuracy is usually assessed using the Pearson correlation coefficient between the predicted values and actual values. However, models constructed using the training group typically provide poor predictive effects in test groups, which may be caused by overfitting. The enhancement of the predictive ability of a model in the test group requires the screening of optimum model parameters and the use of cross-validation methods

to assess the actual predictive ability of the model, followed by the prediction of target traits of all possible combinations by utilizing the genotype data and relevant information, such as environmental factors, provided by the test group, so as to achieve the purposes of prediction and screening.

The analysis of data on the relevant literature is as follows. From the distribution of papers by country or region (Table 1.2.9), it can be seen that the main contributors of core papers were Germany, the USA, and the Netherlands. An identical ranking was observed for the top three countries/regions with the highest number of paper citations. From the distribution of papers by research institution (Table 1.2.10), it can be seen that the Wageningen University and Research (the Netherlands), University of California, Davis (USA), and

University of Paris-Saclay (France) shared the top spot. The network diagram of inter-country/regional collaborations (Figure 1.2.5) shows that inter-country collaborations were common, with close collaborative relationships existing among Germany, the USA, the Netherlands, and France. From the network diagram of collaborations among the major contributing institutions (Figure 1.2.6), it can be seen that collaborative relationships existed among all institutions. The main contributors of core paper citations were the USA and China (Table 1.2.11). From the list of the major core paper citation-contributing institutions (Table 1.2.12), it can be seen that the Chinese Academy of Sciences was far ahead of all other institutions. The average core paper citation year of these institutions was 2017.

Table 1.2.9 Countries or regions with the greatest output of core papers on “genomic selection breeding of crops”

No.	Country/Region	Core papers	Percentage of core papers	Citations	Percentage of citations	Citations per paper
1	Germany	4	66.67%	152	87.36%	38.00
2	USA	4	66.67%	132	75.86%	33.00
3	Netherlands	3	50.00%	115	66.09%	38.33
4	France	3	50.00%	47	27.01%	15.67
5	Australia	1	16.67%	85	48.85%	85.00
6	Saudi Arabia	1	16.67%	85	48.85%	85.00
7	Israel	1	16.67%	19	10.92%	19.00
8	Austria	1	16.67%	11	6.32%	11.00
9	Norway	1	16.67%	11	6.32%	11.00

Table 1.2.10 Institutions with the greatest output of core papers on “genomic selection breeding of crops”

No.	Institution	Core papers	Percentage of core papers	Citations	Percentage of citations	Citations per paper
1	Wageningen Univ & Res	2	33.33%	96	55.17%	48.00
2	Univ Calif Davis	2	33.33%	30	17.24%	15.00
3	Univ Paris Saclay	2	33.33%	36	20.69%	18.00
4	Brigham Young Univ	1	16.67%	85	48.85%	85.00
5	Christian Albrechts Univ Kiel	1	16.67%	85	48.85%	85.00
6	King Abdullah Univ Sci Tech	1	16.67%	85	48.85%	85.00
7	Univ Melbourne	1	16.67%	85	48.85%	85.00
8	Wageningen UR	1	16.67%	85	48.85%	85.00
9	Washington State Univ	1	16.67%	85	48.85%	85.00
10	Commissariat Energie Atom & Energies Alternat	1	16.67%	19	10.92%	19.00



Figure 1.2.5 Collaboration network among major countries or regions in the engineering research front of “genomic selection breeding of crops”

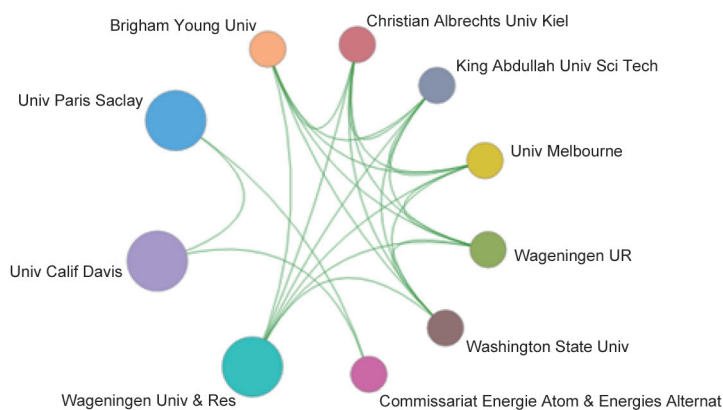


Figure 1.2.6 Collaboration network among major institutions in the engineering research front of “genomic selection breeding of crops”

Table 1.2.11 Countries or regions with the greatest output of citing papers on “genomic selection breeding of crops”

No.	Country/Region	Citing papers	Percentage of citing papers	Mean year
1	USA	50	29.59%	2017.9
2	China	31	18.34%	2017.9
3	Australia	19	11.24%	2018.0
4	Germany	16	9.47%	2017.9
5	UK	12	7.10%	2018.1
6	France	9	5.33%	2017.9
7	Saudi Arabia	7	4.14%	2018.0
8	Netherlands	7	4.14%	2018.1
9	Denmark	6	3.55%	2017.5
10	South Korea	6	3.55%	2018.0

Table 1.2.12 Institutes with the greatest output of citing papers on “genomic selection breeding of crops”

No.	Institutions	Citing papers	Percentage of citing papers	Mean year
1	Chinese Acad Sci	12	21.43%	2017.9
2	Univ Copenhagen	5	8.93%	2017.4
3	Univ Illinois	5	8.93%	2017.8
4	Univ Calif Davis	5	8.93%	2017.6
5	Univ Paris Saclay	5	8.93%	2017.8
6	J Craig Venter Inst	4	7.14%	2018.0
7	Univ Tasmania	4	7.14%	2018.0
8	King Abdullah Univ Sci & Technol	4	7.14%	2017.8
9	Donald Danforth Plant Sci Ctr	4	7.14%	2018.0
10	Michigan State Univ	4	7.14%	2017.8

2 Engineering development fronts

2.1 Trends in the top 10 engineering development fronts

The top 10 engineering development fronts in the agriculture field can be classified into three categories: (1) Ground breaking development front. This includes “gene editing techniques for agricultural organisms” of agricultural bioengineering. (2) Emerging development fronts. These include “prevention and remediation of heavy metal pollution in soil” of resource ecology, “intelligent agricultural equipment” of agricultural engineering, “utilization of forest-produced biomass” of forestry science, and “crop DNA sequence and genome analysis” of crop science. (3) In-depth established development fronts. These include “sustainable plant protection technologies” of plant protection, “animal health management systems” of animal science, “selective breeding of new hybrid crop cultivars” of crop science, “precision cultivation technologies” of crop science, and “effective fry cultivation” of fisheries science.

The numbers of patent disclosures for these fields ranged from 100 to 300, the average number of citations of the patents were within the range 10 to 20, and the mean year of patent disclosure was 2014 (Table 2.1.1). In particular, the number of citations of patents related to gene editing techniques for agricultural organisms showed a rapid increase prior to 2017, which is opposite to the trends showed by patents related to other fronts (Table 2.1.2).

The top 10 engineering development fronts are briefly described below.

(1) Sustainable plant protection technologies

This is an in-depth established development front. Pesticide pollution refers to the excessive use of pesticides and the inappropriate selection of types and application timings of pesticides. Besides resulting in pesticide residue limits in agricultural products being exceeded and posing a serious threat to human health, pesticide pollution also eliminates certain predatory and parasitic natural enemies of crop pests, which upsets the natural balance between pests and their natural enemies, causes pest outbreaks and eliminates pollinating insects, ultimately affecting crop production. Moreover, excessive pesticide movement into soil and water bodies through rainfall, agricultural soil leachates and discharges causes damage to ecosystems and may also result in the development of resistance of pest to chemical pesticides. In view of these issues, the adoption of sustainable control techniques are being encouraged. These are environmentally-friendly agricultural techniques that utilize a combination of biological, physical, agricultural prevention and treatment techniques, and scientific fertilizer application methods for disease and pest prevention and control in crops.

(2) Gene editing techniques for agricultural organisms

This is a ground breaking development front. Gene editing techniques refer to techniques that perform site-specific modifications or changes to the genome and transcriptional products of organisms. Since the early 2000s, genome editing techniques have developed rapidly and led to the successive

Table 2.1.1 Top 10 engineering development fronts in agriculture

No.	Engineering development front	Published patents	Citations	Citations per patent	Mean year
1	Sustainable plant protection technologies	225	3129	13.91	2014.8
2	Gene editing techniques for agricultural organisms	198	3301	16.67	2015.9
3	Prevention and remediation of heavy metal pollution in soil	178	1927	10.83	2014.4
4	Intelligent agricultural equipment	106	1382	13.04	2014.7
5	Utilization of forest-produced biomass	122	1343	11.01	2014.4
6	Animal health management systems	121	2517	20.80	2014.5
7	Effective fry cultivation	131	689	5.26	2014.2
8	Selective breeding of new hybrid crop cultivars	269	4330	16.10	2014.5
9	Precision cultivation technologies	102	1659	16.26	2014.3
10	Crop DNA sequence and genome analysis	246	5158	20.97	2014.6

Table 2.1.2 Annual number of core patents published for the top 10 engineering development fronts in agriculture

No.	Engineering development front	2013	2014	2015	2016	2017	2018
1	Sustainable plant protection technologies	59	50	32	45	35	4
2	Gene editing techniques for agricultural organisms	8	19	37	63	70	1
3	Prevention and remediation of heavy metal pollution in soil	48	46	56	20	7	1
4	Intelligent agricultural equipment	25	22	28	19	11	1
5	Utilization of forest-produced biomass	36	38	27	11	10	0
6	Animal health management systems	41	28	20	20	11	1
7	Effective fry cultivation	42	36	44	9	0	0
8	Selective breeding of new hybrid crop cultivars	75	61	65	50	17	1
9	Precision cultivation technologies	41	24	17	11	7	2
10	Crop DNA sequence and genome analysis	65	67	50	39	21	4

emergence of four novel genome editing tools, namely ZFNs, TALENs, MGNs, and CRISPR/Cas9. Genome editing techniques enable accurate, targeted modifications of specific sites in the genome of organisms, and artificial alterations of genetic information. In particular, the CRISPR/Cas9 system developed in 2013 is a novel technique that enables targeted gene editing. It possesses several advantages over other genome editing tools, including greater ease of operation, lower cost, higher efficiency, and higher targeting accuracy, and has demonstrated great potential in the fields of basic biological research, gene therapy, and genetic modification. Notably, TALENs and CRISPR/Cas9 were listed among the *Science* magazine top 10 scientific breakthroughs of 2012 and 2013, respectively.

(3) Prevention and remediation of heavy metal pollution in soil

This is an emerging development front. Heavy metal pollution of agricultural crops, which directly impacts agricultural production, food security and environments essential for human survival, has become one of the major ecological environmental issues faced by countries worldwide. Heavy metals have a density of at least $4.5 \text{ g}\cdot\text{cm}^{-3}$ and include arsenic (a metalloid), cadmium, chromium, lead, and mercury. Environmental pollution caused by heavy metals or heavy metal compounds is known as heavy metal pollution, which largely arises from human activities, such as mining, exhaust emissions, sewage irrigation and the use of products with heavy metal content that exceed regulatory limits. In particular, heavy metal pollution in agricultural land is

highly elusive. As heavy metals have high toxicities, complex chemical behaviors, adverse ecological effects and extended persistence in soil, they can be absorbed by crops, which leads to their entry into food chains, or migration into water bodies and the atmosphere, thereby posing a severe threat to the survival and sustainable development of human populations. The prevention and remediation of heavy metal pollution in soil produces certain effects on the growth, development, yield, quality, and physiological and biochemical metabolic pathways of crops. There are two main types of heavy metal remediation techniques, with the first being the direct removal of heavy metal-polluted soil and the second being the alteration of the forms in which heavy metals exist in soil to reduce their activity, transferability and bioavailability. At present, remediation methods for heavy metal-polluted soils include physical, chemical, electrochemical and biological remediation. As the heavy metal pollution of agricultural land adversely affects ecological environments, food security and limits agricultural development, it has attracted widespread attention and substantial research efforts in relevant fields, such as environmental science.

(4) Intelligent agricultural equipment

This is an emerging development front. Intelligent agricultural equipment, which were developed based on a combination of advanced manufacturing technologies, information technologies, and artificial intelligence technologies, are important tools in the upgrade and modification of the current agricultural machinery manufacturing industry and the realization of the automation and intelligentization of agricultural production processes. Therefore, they represent a major direction in the development of high-end agricultural equipment manufacturing industries around the world. At present, the focus areas in the intelligentization of control technologies for agricultural equipment are: 1) the in-depth investigation of informatization technologies such as transducers/sensors, communication systems, image processing and computer visuals, as well as the broadening of applications of these technologies, such as the use of transducers/sensors in the control of vehicle steering, the use of level control in the raising and lowering of ground operation parts of vehicles, and the electrohydraulic control of vehicle position and pressure; 2) image sensor monitoring systems based on plant/crop characteristics and the ripeness of agricultural products such as fruits and vegetables, and thinning and weed removal devices with a combination of

image processing and visual sensing functions (automated visual monitoring systems); and 3) collaborative control of harvesters and grain carts in multi-machine collaborative operations through the use of visual and image processing technologies (stereo vision systems), including the radio control of harvester loads from granaries, grain conveying controllers that can control conveying speeds, and the improvement of the threshing mechanism of harvesters and performance of grain collectors. Besides satisfying the different levels of current needs for agricultural equipment, future research on intelligent agricultural equipment will mainly be focused on the digital design and simulation systems of intelligent agricultural equipment, testing platforms of intelligent equipment, microelectromechanical systems, agricultural transducers/sensors, agricultural robots, intelligent navigation control technologies and the integration of advanced technologies, such as the IoT, big data, cloud computing, and cloud services, into the design of intelligent agricultural equipment.

(5) Utilization of forest-produced biomass

This is an emerging development front. There are two main sources of utilizable raw materials in forestry biomass: 1) solid biofuels mainly originating from waste materials of the forestry processing industries, such as the wood panel industry and paper industry; and 2) energy forests established based on the development layout of the biomass energy industry, in which trees with fast growing rates, high calorific values and high oil content are grown to ensure a steady production and supply of raw materials. At present, biomass energy development technologies are mainly concentrated in the areas of gasification, compressed fuels, combustion for power generation, the production of ethanol fuel and the production of biodiesel. In particular, the technologies of gasified biomass fuel production and biomass compression are relatively mature and are considered part of established research. High-efficiency direct combustion for power generation, which is regarded as the most feasible biomass utilization method, represents a key direction in future research.

(6) Animal health management systems

This is an in-depth established development front. Healthy breeding practices provide assurance of animal health, enhanced animal productivity and animal product safety. Animal health management is a key link in the promotion of animal health. It includes the monitoring and assessment

of animal health for the timely discovery of poor health in animals, and the identification and resolution of factors affecting animal health including breeding, nutrition, disease, environment and production, so as to ensure the health of domestic animals. Animal health management is usually manifested by the establishment of a scientific and reasonable animal health management system by combining the current theories and data of the various links in animal breeding with knowledge discovery and data mining methods. In general, animal health management systems mainly encompass health assessment criteria, health promotion methods, disease diagnosis knowledge, disease prevention and treatment methods, veterinary drug compatibility knowledge, and criteria for the use of veterinary drugs. In recent years, traceability information systems for animal production and animal products have developed rapidly. Through the utilization of technologies, such as network communication, QR codes, radio-frequency identification, embedded equipment, smart cards and databases, the various links of animal breeding, including production sites, production processes, feed formulas, immunity, post-slaughter quarantines and product circulation, can be strengthened. The main business data of animals and animal products are then recorded and tracked throughout the entire animal production process for the achievement of whole-process monitoring and tracking management, starting from ear tag manufacturing to ear tag allocation, animal breeding, circulation, and finally to the slaughtering and sale of animal products. The application of 5G-enabled IoT will further enhance the animal health management and product service systems of large-scale animal breeding.

(7) Effective fry cultivation

This is an in-depth established development front. Developments in the proliferation and breeding of marine fishes are the fundamental measures and strategies for the reasonable development and utilization of marine fish resources and sustainable development of the fisheries industry. Besides adequate pre-cultivation preparations, reasonable fry stocking, suitable rearing methods and appropriate daily management, effective fry cultivation may also involve the adoption of the latest biological technologies and methods to achieve cutting-edge development. Novel technologies are mainly focused on the induction of gonadal development and maturation, improvement of oocyte quality,

ovulation and spawning, advancement of the timings of sexual maturity and sex change, regulation of marine cultivated fishes to enable all-year-round gonadal development, gonadal maturation and spawning, improvement of fry cultivation to enhance survival and growth rates, and adoption of genetic engineering technologies to produce various hormones and neuropeptides that promote fish reproduction and growth. Specific techniques include the construction of detailed whole-genome maps of target genotypes, sex control techniques, technologies for the scaled-up production of triploids, and establishment of unisexual sperm banks. In addition, technologies for the production of hyperestrogenic fry, cultivation of high-yield and disease-resistant genotypes, sex control, and crossbreeding have also been utilized for the cultivation of new genotypes of aquatic organisms.

(8) Selective breeding of new hybrid crop cultivars

This is an in-depth established development front. An inbred line of a certain crop refers to a population with identical traits produced from the continuous inbreeding of a single strain. Heterosis achieved from the crossing of inbred lines with high combining ability provides a key route for the substantial enhancement of crop yield, improvement of crop quality, strengthening of stress resistance, and increase in crop adaptability. With the rapid development of cell biology and molecular biology, parent plant selection by plant breeders does not only involve considerations regarding the complementation of agronomic trait advantages, closeness of relationships, combining ability, heritability and resistance, but also involves the screening of parent plants by utilizing genomic in-situ hybridization and molecular marker techniques. By combining genome sequencing techniques with trackable and detectable molecular markers closely linked to the target genes in the genome (e.g., RFLPs, RAPDs, AFLPs, SSRs and SNPs), the efficiency of parental material recombination and screening can be enhanced. The investigation and utilization of target traits at the molecular level by plant breeders also increases the accuracy of breeding value prediction and heterosis effects. Major directions in basic research on the utilization of heterosis are: 1) methods to achieve maximum development and the utilization of heterosis through the use of germplasm resources; 2) in-depth investigation of heterotic populations and the identification of heterotic populations at the molecular level; 3) screening of heterosis related to yield factors for the identification of

QTL; and 4) investigation of populations suitable for heterosis breeding.

(9) Precision cultivation technologies

This is an in-depth established development front. Past agricultural development has mainly been dependent on human labor and has many disadvantages, including long duration, high costs and low efficiency. With advancements in scientific technologies, precision cultivation technologies have emerged and have developed steadily. Such technologies, which have propelled agriculture into the digital and information age, accelerated crop cultivation processes, and reduced the consumption of manpower and material resources, represent a key direction of agriculture development in the twenty-first century. In intelligent precision cultivation technology systems for production regions, greenhouses or plant factories, precision cultivation IoT systems are established to enable the acquisition of big data and characteristic nodule videos of monitored parameters through real-time, whole-process periodic comparisons for the monitoring of cultivation and construction of parameter models for precision cultivation control. Real-time sensor-based monitoring and the processes control parameter iteration are used to construct intelligent expert systems and achieve the purpose of intelligentized precision cultivation. The precision crop cultivation IoT system consists of four major components, namely, on-site infrastructure, equipment monitoring and control, data transfer and storage, and cloud computing platform application and management. It contributes to the achievement of agricultural production under the conditions of intelligent agriculture. The adoption of these novel technologies can effectively reduce labor intensity, enhance production efficiency, and increase crop quality and yields.

(10) Crop DNA sequence and genome analysis

This is an emerging development front. Crop DNA sequence and genome analysis is a basic research area for the realization of transgenic crop breeding, genomic selection breeding of crops, and molecular design breeding. At present, the commonly used technologies are DNA microarrays and massively parallel signature sequencing; other techniques include the serial analysis of gene expression and digital gene expression profiling by expressed sequence tag sequencing. Continuously evolving genome analysis methods facilitates the rapid development of molecular breeding technologies for crops and mainly serve the following

roles. 1) The testing of new transgenic cultivars, i.e., the utilization of modern biological technologies for the artificial isolation, recombination, introduction, and integration of desired target genes into the genome of organisms, so as to improve the original traits or confer new superior traits to the organisms. Besides introducing new exogenous genes, transgenic technologies can also be used to alter the genetic characteristics of organisms through methods such as gene processing, gene knockout and genetic screening to obtain the desired traits. 2) In-depth analyses of gene expression profiles, which are beneficial toward the elucidation of molecular mechanisms of heterosis formation.

2.2 Interpretations for three key engineering development fronts

2.2.1 Sustainable plant protection technologies

Research on plant protection is currently directed toward the development of safe, sustainable and environment-protecting technologies. Novel plant protection technologies include ecological comprehensive prevention and control technologies; prevention and control measures for harmful organisms; innovations in immunoregulators for plants; and the development, management, and application of environmentally-friendly pesticides. (1) Ecological regulation measures that are mainly adopted from a macroscopic perspective and include the selective breeding and promotion of disease and pest-resistant cultivars, optimization of crop layouts, breeding of healthy seed and seedlings, and improvement of water and fertilizer management. These measures are combined with biodiversity regulation techniques and methods for the protection and utilization of natural enemies of pests, such as farmland eco-engineering, grass covers in orchards, intercropping, and natural enemy-trapping belts, so as to modify the sources and breeding environments of diseases and pests and artificially strengthen natural pest control properties as well as disease and pest resistance in crops. (2) Biological prevention and control techniques developed that promote the application of crop protection agents, such as *Trichogramma*, predatory mites, *Metarhizium* spp., *Beauveria* spp., microsporidia, *Bacillus thuringiensis*, *Bacillus cereus*, *Bacillus subtilis* and nucleopolyhedrosis virus, as well as mature techniques such as poultry herding and rice-duck/rice-shrimp co-culture

systems, key biological prevention and control techniques that involve pest control using pests, mite control using predatory mites, pest control using fungi, and fungi control using fungi. Biological prevention and control techniques also include the active development of application techniques for biological and biochemical formulations, such as botanical pesticides, agricultural antibiotics, and plant immunity inducers. (3) Physicochemical trapping techniques actively developed that use and promote insect pheromones (e.g., sex attractants and acrasin), insect light traps and sticky insect traps (yellow and blue) for the control of pests on crops such as vegetables, fruit trees, and tea plants, other physicochemical trapping techniques, such as plant traps, bait traps, insect-shielding nets, and pest-repelling silver-gray film. (4) Scientific pesticide use techniques that emphasize the following areas: the development and application of effective, low-toxicity, low-residue, environmentally-friendly pesticides; optimization and integration of supporting techniques, such as pesticide rotations, pesticide alternations, and precise and safe pesticide usage methods; strengthening of pesticide resistance monitoring and management; promulgation and standardization of knowledge on pesticide use; and strict adherence to safety intervals for pesticide use. Effective, low-risk pesticides must possess a high activity toward target organisms; low dose per unit area; show non-toxicity toward crops, humans, livestock, and beneficial organisms; and exhibit good biodegradability with nontoxic degradation products.

The major contributing countries/regions of patent disclosures for this focal point were Japan, Germany, the USA, China,

and Switzerland (Table 2.2.1). In particular, the number of citations of Japanese patents exceeded the total combined number of citations for Germany, the USA, China and Switzerland with the average number of citations of Japanese patents being 20.54, i.e., around twofold the number of patent citations for other countries or regions. Among the major institutions with the greatest output of core patents (Table 2.2.2), Bayer Cropscience AG of Germany had contributed a significant number of published patents, while the Sumitomo Chemical Co., Ltd. of Japan shows the most citations per patent among the institutions. Other than between Japan and the UK, collaborative relationships for this engineering development front could be found among the major countries and regions (Figure 2.2.1). There were rare collaborations between major institutions (Figure 2.2.2).

2.2.2 Gene editing techniques for agricultural organisms

Gene editing techniques refer to techniques that perform site-specific modifications or changes to the genome and transcriptional products of organisms. Such techniques involve the cutting of genomic DNA at specific sites by restriction endonucleases to generate DSBs. This induces DNA repair via NHEJ or homology-directed repair (HDR), which are major DNA repair mechanisms, thereby achieving the genetic modification of target genes. NHEJ is the main pathway of DNA repair in prokaryotic genomes. It enables the religation of broken DNA ends with or without limited homologous sequences. However, as NHEJ is an error-prone mechanism,

Table 2.2.1 Countries or regions with the greatest output of core patents on “sustainable plant protection technologies”

No.	Country/Region	Published patents	Percentage of published patents	Citations	Percentage of citations	Citations per patent
1	Japan	74	32.89%	1520	48.58%	20.54
2	Germany	58	25.78%	578	18.47%	9.97
3	USA	40	17.78%	438	14.00%	10.95
4	China	32	14.22%	318	10.16%	9.94
5	Switzerland	15	6.67%	139	4.44%	9.27
6	UK	3	1.33%	123	3.93%	41.00
7	France	2	0.89%	23	0.74%	11.50
8	Netherlands	2	0.89%	12	0.38%	6.00
9	India	1	0.44%	12	0.38%	12.00
10	Belgium	1	0.44%	7	0.22%	7.00

Table 2.2.2 Institutions with the greatest output of core patents on “sustainable plant protection technologies”

No.	Institution	Country/Region	Published patents	Percentage of published patents	Citations	Percentage of citations	Citations per patent
1	FARB	Germany	52	23.11%	530	16.94%	10.19
2	SUMO	Japan	24	10.67%	748	23.91%	31.17
3	DOWC	USA	24	10.67%	340	10.87%	14.17
4	SYGN	Switzerland	16	7.11%	252	8.05%	15.75
5	NIPY	Japan	16	7.11%	201	6.42%	12.56
6	NISC	Japan	13	5.78%	349	11.15%	26.85
7	BADI	Germany	8	3.56%	68	2.17%	8.50
8	SNCM	China	7	3.11%	52	1.66%	7.43
9	NIPS	Japan	7	3.11%	46	1.47%	6.57
10	SNFI	China	5	2.22%	35	1.12%	7.00

FARB: Bayer Cropscience AG; SUMO: Sumitomo Chemical Co., Ltd.; DOWC: Dow Agrosciences LLC; SYGN: Syngenta Participations AG; NIPY: Nihon Nohyaku Co., Ltd.; NISC: Nissan Chemical Industries Ltd.; BADI: BASF SE; SNCM: Sinochem Agro Co., Ltd.; NIPS: Nippon Soda Co., Ltd.; SNFI: Merial Inc.

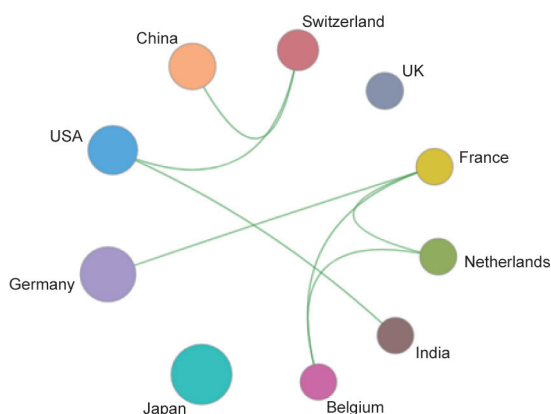


Figure 2.2.1 Collaboration network among major countries or regions in the engineering development front of “sustainable plant protection technologies”

and nucleotide insertions/deletions commonly occur during the repair process, causing frameshift mutations which lead to the achievement of gene knockout. In the presence of homologous sequences, the probability of HDR occurring at DSBs is increased. The HDR pathway enables the achievement of site-specific gene editing or gene knockin through the replacement or recombination of homologous DNA at DSBs for the recovery or alteration of genetic information. Both NHEJ and HDR are dependent on the generation of DSBs; however, naturally occurring DSBs are extremely rare in genomes. Therefore, the key issue in genetic editing in animals is the induction of DSBs at specific sites. Novel genome editing techniques, such as ZFNs, TALENs, MGNs, and the CRISPR/

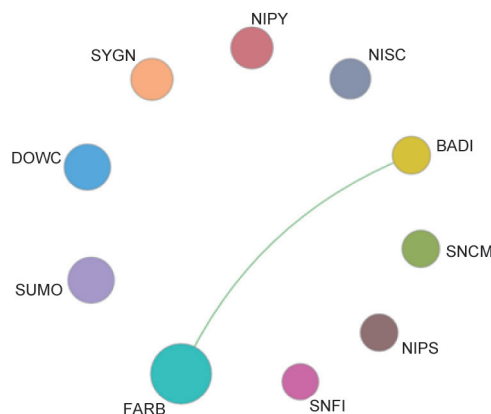


Figure 2.2.2 Collaboration network among major institutions in the engineering development front of “sustainable plant protection technologies”

Cas9 system, enable the targeted cutting of genomes for the generation of DSBs, which triggers DNA repair in cells by NHEJ or HDR, and ultimately achieves effective and accurate genome editing in various types of organisms.

The major contributing countries of patent disclosures for this focal point were the USA and China (Table 2.2.3), with the number of patent citations being substantially higher for US patents than other patents. Although relatively few patent disclosures were contributed by Lithuania, Germany, France, Switzerland and the UK, the proportions of patent citations were relatively high for these countries. The average number of citations of US patents was 23, which was threefold the

number of citations for Chinese patents. It was also observed that a close collaborative relationship existed between the USA and Switzerland (Figure 2.2.3). From the distribution of core patents among the various organizations (Table 2.2.4), it can be seen that the Harvard University, Massachusetts Institute of Technology, and Broad Institute (USA) occupied the top three spots. Notably, among the patents related to genome editing techniques, the number of applications for CRISPR/Cas9 patents was substantially higher than that of patents related to ZFNs, TALENs, and MGNs, and the patent applications for these techniques mainly pertained to the applied use of the techniques. These three US organizations have collaborated closely to promote

the wide application of genome editing techniques in the field of biomedical research (Figure 2.2.4). In the fields of animal improvement and agricultural crop research, the number of Chinese patents has increased at a relatively high rate. China Agricultural University has contributed a great number of patents on animal breeding-related gene editing techniques, which have focused on the development and utilization of pig, goat, and cattle disease models. Other Chinese institutions, including the Institute of Genetic and Developmental Biology, China Academy of Sciences, and Chinese Academy of Agricultural Sciences, have filed a great number of patent applications related to the genetic breeding of agricultural crops.

Table 2.2.3 Countries or regions with the greatest output of core patents on “gene editing techniques for agricultural organisms”

No.	Country/Region	Published patents	Percentage of published patents	Citations	Percentage of citations	Citations per patent
1	USA	90	45.45%	2095	63.47%	23.28
2	China	79	39.90%	623	18.87%	7.89
3	Germany	6	3.03%	104	3.15%	17.33
4	Japan	5	2.53%	40	1.21%	8.00
5	France	4	2.02%	94	2.85%	23.50
6	Switzerland	4	2.02%	74	2.24%	18.50
7	Lithuania	3	1.52%	331	10.03%	110.33
8	South Korea	3	1.52%	37	1.12%	12.33
9	UK	2	1.01%	69	2.09%	34.50
10	Netherlands	2	1.01%	24	0.73%	12.00

Table 2.2.4 Institutions with the greatest output of core patents on “gene editing techniques for agricultural organisms”

No.	Institutions	Country/Region	Published patents	Percentage of published patents	Citations	Percentage of citations	Citations per patent
1	HARD	USA	17	8.59%	475	14.39%	27.94
2	MASI	USA	10	5.05%	501	15.18%	50.10
3	BROD	USA	7	3.54%	451	13.66%	64.43
4	SPHS	China	7	3.54%	55	1.67%	7.86
5	REGC	USA	6	3.03%	94	2.85%	15.67
6	AGIL	USA	5	2.53%	54	1.64%	10.80
7	CARI	USA	4	2.02%	169	5.12%	42.25
8	ALNY	USA	4	2.02%	74	2.24%	18.50
9	UCAG	China	4	2.02%	53	1.61%	13.25
10	WHED	USA	4	2.02%	47	1.42%	11.75

HARD: Harvard College; MASI: Massachusetts Institute of Technology; BROD: Broad Institute, Inc.; SPHS: 2nd People’s Hospital of Shenzhen; REGC: University of California; AGIL: Agilent Technologies, Inc.; CARI: Caribou Biosciences, Inc.; ALNY: Alnylam Pharmaceuticals, Inc.; UCAG: China Agricultural University; WHED: Whitehead Institute for Biomedical Research.

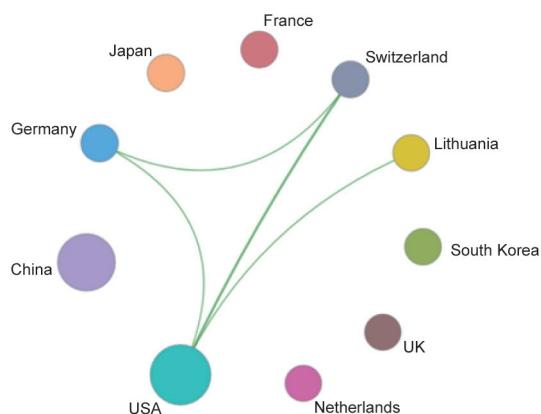


Figure 2.2.3 Collaboration network among major countries or regions in the engineering development front of “gene editing techniques for agricultural organisms”

2.2.3 Prevention and remediation of heavy metal pollution in soil

Heavy metal pollution in soil directly impacts crop growth and development, thereby affecting yield formation in crops and agricultural products. It is characterized by the poor mobility and extended persistence of pollutants in soil, as well as elusiveness and irreversibility. Heavy metal pollutants may also enter the human food chain through crops and could severely impact food safety and human health. In many countries and regions, such as the USA, the EU, Japan, and China, active deployment and research efforts have been devoted toward the development of remediation technologies for soils with heavy metal pollution. At present, the key methods for the remediation of heavy metals in soil include biological, physical, and chemical remediation, and various combinations of these. Japanese-sponsored research projects on soil pollution remediation have mainly focused on measurement methods for heavy metal pollutants in soil, soil remediation technologies (biological and chemical), soil remediation mechanisms, and assessments of the environmental impacts of soil pollution. To tackle the increasingly severe issue of heavy metal pollution, South Korea has devoted greater effort to the development of soil washing and electrokinetic remediation technologies. In Taiwan, China, in-situ combined remediation technologies have mainly been adopted in recent years. The USA holds the leading position in the application of remediation technologies in polluted sites, with projects of the Superfund program mostly employing in-situ soil vapor extraction,

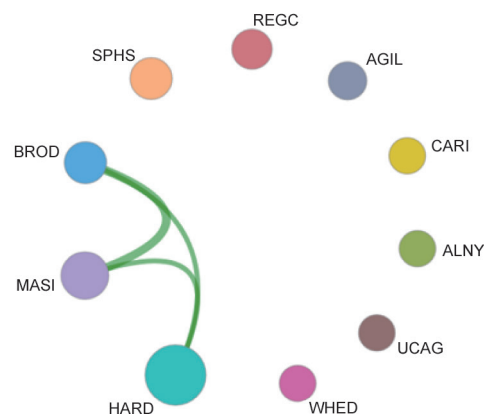


Figure 2.2.4 Collaboration network among major institutions in the engineering development front of “gene editing techniques for agricultural organisms”

ex-situ solidification/stabilization and ex-situ incineration technologies. In the EU, the most common method of soil remediation is the excavation and landfill disposal of polluted soil. With increases in the costs of supervising landfill operations and other related costs, enhancements in ex-situ remediation technologies and the combined use of in-situ and ex-situ remediation technologies and pollution remediation methods have been made. In summary, there are two main types of remediation technologies for heavy metal pollution, with the first being the direct removal of heavy metal-polluted soil and the second being the alteration of the forms in which heavy metals exist in soil to reduce their activity, transferability and bioavailability. The specific remediation technologies can be further classified as (1) in-situ stabilization technologies, including in-situ chemical passivation, microbial adsorption, and plant fixation; (2) engineering remediation technologies, including phytoremediation, soil replacement, dilution by deep soil mixing, and soil washing; (3) agricultural control measures, including water and fertilizer management, the regulation of soil pH, and intercropping; and (4) plant prevention and control technologies, including the physiological prevention and control by plant foliage, application of low-absorption crop genotypes, genetic engineering, and adjustment of crop planting structures.

China accounted for the vast majority of patent disclosures for this focal point, while the USA and Japan have also contributed certain proportions of disclosures. Only seven countries have disclosed relevant patents. Although China has contributed a large number of patents, the average number

of citations of Chinese patents was only about half that of US patents (Table 2.2.5). From the distribution of core patents among various organizations, it can be seen that Suntime Environmental Remediation Co., Ltd. in Jiangsu, China had contributed a significant number of patents, while the

distribution of patents among organizations of other countries was relatively sparse (Table 2.2.6). The network diagrams of inter-country/regional collaborations and inter-organization collaborations indicate the absence of collaborations in the development of relevant patents (Figures 2.2.5 and 2.2.6).

Table 2.2.5 Countries or regions with the greatest output of core patents on “prevention and remediation of heavy metal pollution in soil”

No.	Country/Region	Published patents	Percentage of published patents	Citations	Percentage of citations	Citations per patent
1	China	154	86.52%	1592	82.62%	10.34
2	USA	12	6.74%	226	11.73%	18.83
3	Japan	7	3.93%	69	3.58%	9.86
4	South Korea	2	1.12%	12	0.62%	6.00
5	Australia	1	0.56%	14	0.73%	14.00
6	Italy	1	0.56%	8	0.42%	8.00
7	Netherlands	1	0.56%	6	0.31%	6.00

Table 2.2.6 Institutions with the greatest output of core patents on “prevention and remediation of heavy metal pollution in soil”

No.	Institutions	Country/Region	Published patents	Percentage of published patents	Citations	Percentage of citations	Citations per patent
1	CAEM	China	4	2.25%	46	2.39%	11.50
2	USCU	China	3	1.69%	56	2.91%	18.67
3	JIAN	China	3	1.69%	52	2.70%	17.33
4	UYJN	China	3	1.69%	42	2.18%	14.00
5	UYHD	China	3	1.69%	40	2.08%	13.33
6	CRSM	China	3	1.69%	35	1.82%	11.67
7	UYGU	China	3	1.69%	34	1.76%	11.33
8	USJT	China	3	1.69%	33	1.71%	11.00
9	UYHU	China	3	1.69%	24	1.25%	8.00
10	CHAN	China	2	1.12%	33	1.71%	16.50

CAEM: Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences; USCU: Sichuan University; JIAN: Jiangsu Suntime Environmental Remediation Co., Ltd.; UYJN: Jiangnan University; UYHD: North China Electric Power University; CRSM: Institute of Rock and Soil Mechanics, Chinese Academy of Sciences; UYGU: Guangxi University; USJT: Shanghai Jiaotong University; UYHU: Hunan University; CHAN: Changsha Hasky Environmental Protection Science and Technology Development Co., Ltd.

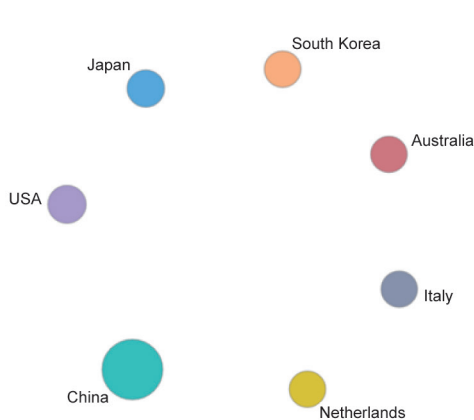


Figure 2.2.5 Collaboration network among major countries or regions in the engineering development front of “prevention and remediation of heavy metal pollution in soil”

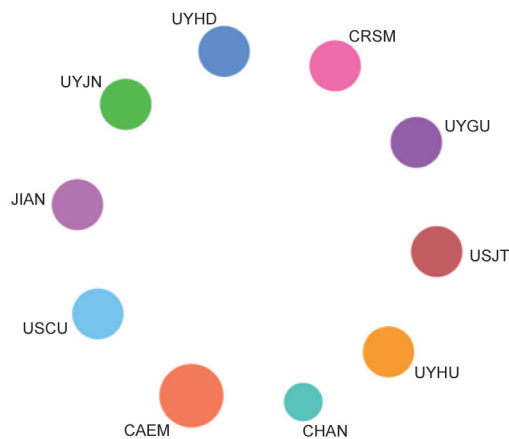


Figure 2.2.6 Collaboration network among major institutions in the engineering development front of “prevention and remediation of heavy metal pollution in soil”

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