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Review

The application of plant biotechnology for non-food uses: The case of China

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Applications of plant biotechnology play increasingly important role in solving food and energy crisis. As the world's largest country by population, China pays special attention to the development of agricultural biotechnology and biomass energy technology. Several excellent review articles have introduced the research progress of China's crop biotech and bioenergy. However, plant biotechnology in China has more diverse applications for non-food uses and environmental sustainability. This paper reviews the recent applications of plant biotechnology in bioenergy, biocontrol, traditional Chinese medicine (TCM), and phytoremediation of polluted environments. The emphasis is mainly focused on the bioconversion of biofuels especially bioethanol and the activated carbon, the survey of energy plants rich in hydrocarbons and carbohydrates, the biocontrol agents (plant epiphytic yeasts) and phytoremediation of heavy metal pollution and water eutrophication. Moreover, plant biotechnology shows very good efficacy in the identifications of confused medicinal plants, active constituents of TCM and the taxonomic identification of plant diseases and insects. The huge number of biomass residue and abundant plant resources provide vast application perspectives for China's plant biotechnology.

Key words: Biomass energy, biocontrol agents, environmental pollution, phytoremediation, plant biotechnology, traditional Chinese medicine.

INTRODUCTION

The prominent problems facing the world today involve frequent natural disasters, food shortage, excessive use of resources, environmental pollution, etc., which severely restrict sustainable development of human society. Governments and scientific communities around the world have made great effort to deal with these issues. Application and rapid development of plant biotechnology play ever-increasing roles in grain yield increase, development of alternative energy, conservation and utilization of plant resources, and phytoremediation of environmental pollution (Chen and Qu, 2002; Han et al., 2007; Davies et al., 2010), thus it brings opportunities to meet various demands. More and more countries have being carrying out research and application of plant biotechnology (Runge and Ryan, 2004).

China is the largest developing agricultural country (Zhou et al., 2011), with the largest population. Plant biotechnology is widely applied for food and non-food uses, as well as in environmental sustainability. Food security is of priority for China. Agricultural biotechnology is a core field of plant biotechnology researches. Chinese government attaches great importance to the field of agricultural biotechnology. The total investment on agricultural research and development gradually increases by the year (close to the 1% of its agricultural GDP in 2009, 0.8% in 2008 and 0.23% in 1999) (James, 2009). Agricultural biotechnology has always been supported as one of the priorities in the Chinese National High-Tech R&D Program since 1986 (Xu et al., 2007). The research projects mainly include plant tissue culture, genetic engineering, genetic mapping, marker-assistant breeding, etc. Rapid progress is achieved in functional genomics research of dominant crops such as rice, cotton and corn (Qin and Zhu 2007; Wang et al., 2008; Li et al.,

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2009; Luan et al., 2011).

Recent publications systematically reviewed the research progress in China's crop biotechnology (Huang et al., 2002; Xu et al., 2007; Karplus and Deng, 2008). Another research hotspot of plant biotechnology is the bioconversion of plant residues (agricultural residues, forest residues and organic wastes) to biofuels (biogas, bioethanol and biodiesel productions). China has become the largest energy user in the world (IEA, 2010). Recent rapid economic growth of China results in the increasing dependence of imported fuel oil. Diversification of fuel supply is an essential choice for the national security and sustainable development. China is committed to greatly developing biomass energy due to the huge bioenergy potential and the reduction of environmental pollution. It is calculated that total bioenergy potential of the biomass resources for the base years of 2007 to 2008 accounts for about 30.2% of China's energy consumption in 2008 (Zhou et al., 2011). Currently, significant progresses are achieved in energy plant survey (Fu and Huang, 2006; Ma et al., 2007; Wu et al., 2010), bioconversion of biomass resources to biofuels (Qu, 2007; Wu et al., 2010; Guo, 2009; Zhang and Zhao, 2010; Ding et al., 2010), bioenergy potential assessment (Yu and Tao, 2009; Tao et al., 2011; Zhou et al., 2011), etc.

In addition to the aforementioned fields, plant biotechnology has more diverse applications in China, for instance, wild plant resource conservation, identification of confused medicinal plants, botanical pesticide, water eutrophication and heavy metal pollution, etc. However, the majority of the basic research findings are reported in Chinese with or without English abstract, which limits academic exchange and application of new achievements in plant biotechnology to a large extent. In contrast, the minority is reported in English and published in the top international academic journals. Recent review articles systematically reported the research progress of China's plant biotechnology. Whereas, most of these papers mainly focused on the application of plant biotechnology for food use (Huang et al., 2002; Xu et al., 2007; Karplus and Deng, 2008), beyond that, several excellent articles reviewed the application status of bioenergy (Wu et al., 2010), bioethanol development (Tao et al., 2011) and biomass energy assessment (Zhou et al., 2011). However, rapid progress in biomass energy was achieved in the recent two years; the preparation of activated carbon from rice husk ash (An et al., 2011), *Camellia oleifera* shell (Sun et al., 2011) and rubber-seed shell (Sun and Jiang, 2010), etc. Only in the first half year of 2011, more than 30 papers by Chinese scientists have been published in the leading international academic journal- Biomass and Bioenergy.

This paper summarizes the research progress of plant biotechnology for non-food uses. Special attentions are paid to the applications in bioenergy plants, traditional Chinese medicine, biocontrol of plant disease and pest and phytoremediation of soil and water pollution.

BIOTECHNOLOGY OF BIOENERGY PLANTS

Developing new alternative energy has become a consensus of the international community for solving world energy crisis. New energy sources include wind energy, solar energy, bioenergy, geothermal energy, solid waste, hydrogen energy, nuclear energy, etc. Of these, biomass energy has become the fourth largest energy source in the world, accounting for 14% of entire world's energy supply (WEC, 1998). China's government pays special attentions to biomass energy for reducing the country's dependence on scarce fossil fuels and alleviating environmental pollution. In 2005, China's government issued 'The Law of Renewable Energy', followed in 2007 by the 'Medium and Long-term Development Program for Renewable Energy', in which industrialization of biomass electricity, biogas, biomass briquettes and biomass liquid fuels were emphasized (Wu et al., 2010). To date, biomass energy has become the third energy source after coal and oil in China, accounting for about 15% of entire energy consumption. The huge number of biomass residue such as crop and forest residues provide adequate raw materials for biofuel productions. For example, the energy potential of agricultural residues in 2008 was estimated to be 14.7 EJ (Zhou et al., 2011).

In recent years, China has achieved rapid progress in the researches and applications of biofuels, especially the bioethanol and biodiesel. Tao et al. (2011) reviewed the newest research progress of China's bioethanol. This review article discussed the status of China's bioethanol development, fuel supply, demand and distribution of bioethanol expansion. Wu et al. (2010) also introduced the applications of bioethanol, biodiesel, biomass electricity and biogas technology in China. Moreover, several papers published in 2011 reported bioconversion technology of ethanol from inulin (Wang et al., 2011), sugarcane bagasse (Zhao et al., 2011), wheat and barley straws (Li et al., 2011; Xu et al., 2011), etc. Another important application of bioenergy technology is the bioconversion of activated carbon. Crop residue is considered as an important biomass resource for biocarbon production. The dominant crop residues such as rice husk ash and corn stover have been converted successfully to activated carbons (Ouyang et al., 2011; An et al., 2011). In addition, the preparation of activated carbon from rubber-seed shell and *C. oleifera* shell are also highlighted (Sun et al., 2010, 2011).

Indeed, the application of the bioenergy technologies plays important roles in energy supply and the control of environmental pollution. However, bioenergy development in China faces some problems (Wu et al., 2010), such as unstable quality of biodiesel caused by the feedstock, deficient investigation on biodiesel plants and limited amount of ethanol gasoline, etc. China has abundant energy plant resources. About 4,000 vascular plants have the potential to be use as energy plants (Lin et al., 2006). Especially in the oil plants (about 1,500 species) investigated, the seeds of 154 oilseed species have high oil contents (over 40% oil) and 30 species of shrubs or trees are rich in biofuel components (Fu and Huang, 2006; Ma et al., 2007). Chinese researchers have great interest in investigating the energy plants rich in hydrocarbons (*Euphorbia tirucalli* and *Euphorbia lathyris*), carbohydrate (sweet sorghum), starch (cassava), fiber (Chinese silvergrass and fiber sorghum), and lipid (*Jatropha curcas*, *Xanthoceras sorbifolia*, *Pistacia chinensis* and *Tetraena monglica*, etc.), as well as microalgae (Stewart Jr et al., 2009). Four primary energy plants, including Barbados nut, Jerusalem artichoke, sweet sorghum and Chinese silvergrass (*Miscanthus sinensis* Anderss.) are researched deeply for the improvements of their attributes and the cultivation of new cultivars by using tissue culture, molecular markers, genetic transformation, and genome sequencing, etc. For Barbados nut, regeneration systems of the species have been established by genetic engineering and tissue culture (Lu et al., 2003; Qin et al., 2006). Using modern plant biotechnology, the sequences of genes regulating physiological metabolism and stress resistance were obtained successively from the organs (leaves, endosperm, and seedlings, etc.) of Barbados nut (Wang et al., 2007; Liang et al., 2007). Moreover, a full-length cDNA of an acyl-acyl carrier protein thioesterase (JcFATB1) was isolated from Barbados nut (Wu et al., 2009). Proteomic characteristics of Barbados nut endosperm was analyzed in seed germination (Yang et al., 2009). The optimal conditions for *Agrobacterium tumefaciens* mediated genetic transformation of Barbados nut were also examined (Li et al., 2006). In recent years, biodiesel from Jatropha has attracted considerable attention due to its huge potential for biodiesel production, non-edible tree-based oil seeds availability in China and high stress resistance, etc (Wu et al., 2010). In contrast, basic research and application of plant biotechnology are less for other energy plants. To date, only a few other energy plants such as sugarcane (Yao et al., 2004; Zhang et al., 2006; Zhao et al., 2011), sweet potato (Li et al., 2005) and cassava (Qin et al., 2008) can be transformed successfully.

BIOCONTROL OF PLANT DISEASES AND PESTS

China achieves great success in transgenetic pest-resistant crops. This attracts more Chinese scientists to apply plant biotechnology for the control of plant diseases and insects. Biopesticide, especially botanical pesticide, is one of the research hotspots. Plant activator protein isolated from different kinds of fungi can induce plant disease-resistance, increase the crop yield and potentially reduce the negative effects of chemical fungicides on the environment (Liu et al., 2011). Some plant epiphytic yeasts are characterized as biocontrol agents of plant diseases and insects (Wang et al., 2009; Liu et al., 2009; Fu et al., 2010; Liu et al., 2011). These biocontrol agents can be isolated from various plant organs; for example, the yeast strain 0732-1 was isolated from the leaves or flowers of watermelon (Wang et al., 2009), the bacterial strain E1R-j from wheat roots (Liu et al., 2009) and antagonistic bacterial strain B106 from the rhizospheric soil of banana plants (Fu et al., 2010), etc.

A more common use of the biocontrol agents is thespray application or seed treatment; it showed a high efficacy in the control of plant diseases. Su et al. (2007) found that the disease resistance of *Atractylodes macrocephala* was increased obviously by spraying with plant activated protein. Plant activated protein enhanced the commercial quality and yield of *A. macrocephala.* The control efficacy to spot blight was 63.3% and the yield was increased by 129.93%. Moreover, Zhou et al. (2008) studied the expression of activator protein Ap36 in *Bacillus thuringiensis* and the effects of recombined strain on disease resistance. They found that the potato (*Solanum tuberosum*) chips treated with the cultured recombinant strain showed higher resistance to rot disease caused by *Erwinia carotovora* SCG1 than the control. Tissue culture technology is also increasingly applied into botanical pesticide (Gou et al., 2009). South China Institute of Botany, Chinese Academy of Sciences, South China Agricultural University and Yunnan Academy of Forestry successively carried out tissue culture of some insecticidal plants such as Neem and *Pyrethrum cinerariifolium*. Subsequently, Northwest Agriculture & Forestry University carried out wide researches on the tissue culture of insecticidal and fungicide plants, including tobacco, *Catharanthus roseus*, *Tripterygium wilfordii*, *Euphorbia fischeriana* and *Calamus*, etc.

Another application of plant biotechnology is the taxonomic identification of plant diseases and pests and the phyletic evolution. Particularly, modern plant biotechnologies such as random amplification of polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), and SSCP have been applied to the identification and phyletic evolution of *Bursaphelenchus xylophilus,* an important pathogen causing destructive disease of pine trees. For example, Zhang et al. (2002) investigated the intra- and inter-specific variation between *B. xylophilus* and *B. mucronatus* by using mtDNA polymorphism. Their results revealed significant inter-specific variation and lesser intra-specific variation between *B. xylophilus* and *B. mucronatus*. Wu et al. (2005) also analyzed the genetic relationship of inter- and infra-species of *B. xylophilus* and *B. mucronatus* by using RAPD. They divided the investigated *B. xylophilus* into three sub-groups: (1) Chinese and Japanese isolates, (2) Taiwan China and American isolates, (3) Canadian isolate; and *B. mucronatus* into two sub-groups: (1) Japanese, Taiwan China and Jiangsu China isolates, (2) South Korea and

Hunan China isolates. Moreover, Hu and Yang (1995) successfully distinguished *B. xylophilus* and *B. mucronatus* using enzyme electrophoresis.

Planting of transgenetic crops makes great contribution to the goal of food self-sufficient for China. However, large-scale cultivation of transgenetic crops may influence the structures and functions of animal communities in the crop fields and the biological chain of crop-pests-predatory enemies. For example, transgenetic Bt cotton grown in northern China not only influenced population densities of the cotton bollworm, but also caused changes and rearrangements in the arthropod assemblages (Guo, 2007). In contrast, the population quantity of some secondary pests such as sucking bugs, whiteflies and aphids was increased significantly (Cui and Xia, 2000; Liu et al., 2002; Guo, 2007). The population quantity of insect natural enemies such as *Propylaea japonica* was decreased in the transgenetic Bt cotton fields. Moreover, the planting of transgenetic cotton might influence some economic insects such as *Bombyx mori* and *Antheraea pernyi* (Li et al., 2003; Fan et al., 2003; Li et al., 2004), pollinating insects (Liu and Xu, 2003), soil fungi and soil bacteria (Wang et al., 2004; Shen et al., 2004), etc.

TRADITIONAL CHINESE MEDICINE

Traditional Chinese Medicine (TCM) is an ancient medical system with a history of 2000 to 3000 years. The TCM takes a holistic approach to treat diseases and always works on the individual's ability to cure itself naturally and build resistance (Manfred, 1974). This medical system is fundamentally different from that of Western medicine that addresses the disease itself to work directly on the symptoms and local cause, and treats the disease by removing the cause. Herbal therapy is one of the main TCM approaches; it mainly uses combinations of medicinal herbs derived from natural sources (plant organs such as leaves, branches and roots, fungi, and minerals, etc.) to comprehensively treat diseases (TCMWF, 2010). By using the combinations, herbal therapies offer gentle effects and relatively few side-effects (Zhou and Wu, 2006). Herbal therapies are suited to treat chronic and lifestyle-related diseases, allergies, various symptoms caused by a stroke or diabetic neuropathy, various hyperthyroidism symptoms, uncomfortable symptoms in atopic dermatitis and other conditions (TCMWF, 2010).

China has very abundant wild medicinal plant resource. To date, about 11,000 medicinal plant species have been found (Hua et al., 2010). However, the reserves of wild medicinal plants are limited and the cultivated medicinal plants usually have a low quality. Another prominent problem is that the process of TCM internationalization is too slow. Currently the TCM has gained legal status in Australia, Russia, Japan, Korea and some Southeast Asian countries; however, it is not recognized widely by Europe and America. Active constituents of TCM and the related action mechanisms are poorly understood; this may be the main reason leading to TCM non acceptance widely by western countries. Applications of plant biotechnology bring opportunities for large-scale oriented cultivation of medicinal plants, identification of the active constituents and the availability of medical products from transgenetic plants. Moreover, plant biotechnology would play crucial roles in promoting TCM internationalization.

The TCM plant biotechnology started from the 1960s as *vitro* propagation. Chinese researchers have established the fluid propagation systems of *Cephalotaxus fortunei*, *Taxus chinensis*, *Catharanthus roseus*, etc. and the *Agrobacterium tumefaciens*-mediated transformation culture systems of liquorice root, *Radix astragali*, and *Artemisia apiacea*, etc (Hu and Liu, 1999). In recent years, modern plant biotechnology are increasingly applied to TCM for the analysis and identification of active constituents by using DNA genetic markers and electrophoretic technique, the pharmacological research by differential immunodiffusion, and the genetic improvement and the production of secondary metabolism products by gene engineering (Zhang et al., 1997; Sha et al., 2002; Liu et al., 2002). Some confused medicinal plants belonging to *Polygonatum*, *Indigofera*, and *Panax*, etc. have been identified accurately by using RAPD finger printings (Wang et al., 2005), or by the combination of DNA fingerprinting and HPLC fingerprinting (Shi et al., 2009). Zeng et al. (2003) analyzed ribosomal DNA sequences of *Radix puerariae* and its sibling species by PCR based DNA sequencing technique.

Some research interests are focused on the genetic engineering of *R. astragali* hairy root and the (+)-d-cadinene synthase gene transfer and expression of *A. apiacea* hairy root (Wang et al., 2005). Other scientific interests concentrate on new drug development by biochip technique, bioreactor establishment by plant cell engineering and specific production of good medicative parts for TCM by fermentation engineering. Moreover, action mechanisms of active constituents of TCM have also made some progress by using modern plant biotechnology (Li et al., 2004). For example, Liu et al. (1999) found that compound Chinese drug bailong promoted the expression (both mRNA and protein) of cyclin-dependent kinase inhibitor (p16INK4a) obviously in G1 phase cells. In addition to that, they also found that bailong can affect many anticancer genes (including p16INK4a, p21 and Rb genes) and oncogenes (such as c2myc) transcription by regulating cAMP2PKA pathway. Shi et al. (2003) analyzed the anti-Lewis lung carcinoma related target-gene in tumor tissue treated by Wusan Granule by cDNA microarray.

PHYTOREMEDIATION OF AIR, SOIL AND WATER POLLUTION

China’s industrialization and urbanization has led to severe air pollution characterized by high concentrations of sulfur dioxides (SO_2), nitrogen oxides (NO_x), fine particulates in cities (Hao et al., 2007), and the largest carbon dioxide (CO_2) emissions in the world (Netherlands Environmental Agency, 2007). For soil pollution, heavy metal polluted agricultural acreage was about 2.0×10^7 ha, accounting for 20% of total agricultural acreage (Chen and Liao, 2004). Moreover, eutrophication of lakes and reservoirs is also very severe, particularly in the five largest freshwater lakes in China (Jin, 2008), involving the Poyang Lake, the Dongting Lake, the Tai Lake, the Hongze Lake and the Chao Lake. Reduction in environmental pollution is thus, an issue of considerable urgency.

Phytoremediation technologies of environmental pollution attract more and more research interests of Chinese environmentalists and biologists. Phytoremediation is a bioremediation process that uses green plants to remove, transfer, stabilize, and/or destroy pollutants from the environment or render them harmless (Zhou, 2006). Phytoremediation mechanisms mainly involve:

(1) Rhizosphere biodegradation,
(2) Phyto-stabilization,
(3) Phyto-accumulation (also called phyto-extraction),
(4) Hydroponic systems for treating water streams (rhizofiltration),
(5) Phyto-volatilization,
(6) Phyto-degradation, and
(7) Hydraulic control, etc (ITRCWGPWT, 2001).

Since 1950, China begun to study the technologies of cleaning heavy metal and organic polluted soils, however, the related researches significantly fall behind the developed countries of Europe and America (Chu et al., 2005). Herbal hyperaccumulator investigated in China are about 30 species (Zhao and Luo, 2009), including *Pteris cretica*, *Commelina communis*, *Eichhornia crassipes*, *Juncus effusus*, *Oenanthe javanica*, *Ipomoea aquatica*, *Lolium multifolorum*, *Acorus calamus*, *Scirpus tabernaemontani*, etc. However, most researches on hyperaccumulator are mainly focused on the plants absorbing a few species of heavy metals such as Cd (Huang, 1989; Wei and Zhou, 2004; Liu et al., 2003), Zn (Yang and Long, 2002) and Cu (Li et al., 2002). Moreover, Xue and Chen (2003) found that pokeberry root had a high capacity of manganese (Mn) enrichment; this finding filled a void of the selection of Mn enrichment plants in China. For the mechanism research of heavy metal pollution, Zhou (1992) investigated the interaction of absorption and accumulation of Zn and Cd of rice tissues. They found that rice leaves produced a counteraction on the absorption and accumulation of Zn and Cd, whereas its roots produced an additive action on the absorption and accumulation of the two heavy metal irons. Therefore, the author included that the mechanism of combined heavy metal pollution is more complex as compared with single heavy metal pollution. Some scientists pay more attention to phytoremediation technologies of water eutrophication and the selection of aquatic plants. Currently, several internationally recognized aquatic plants for water restoration include *Typha latifolia, Phragmites communis, Vallisneria natans, Eichhornia crassipes, Hydrilla verticillata,* and *Alopecurus pratensis* L. Chinese scientists made some efforts to select the aquatic plants with phytoremediation function of water eutrophication; the aquatic plants investigated involved *J. effusus, S. tabernaemontani, Zizanialatifolia, A. calamus, O. javanica, L. multifolorum,* etc (Wang and Chen, 2001; Huang et al., 2007).

Artificial wetland technique has been used as a method of sewage treatment since 1970s (Yu et al., 2008). In China, this technology began from 1990 and was initiated by South China Institute of Environmental Sciences in combination with Shenzhen Dongsen Water Supply Bureau. Subsequently, Jing et al. (2002) studied the effects of plantation of *Cyperus alternifolius* artificial wetland on sewage and their results show that this artificial wetland removed total nitrogen and phosphorus of sewage by 63.6 and 46.6%, respectively and also reduced chemical oxygen demand (COD) and biochemical oxygen demand (BOD) by 73.8 and 73.7%, respectively. Yue et al. (2002) constructed the *Lolium multiflorum-Reineckia carnea* artificial wetland system with a high removal efficiency of NH_4^+-N (96.43%).

Another phytoremediation technology is the aquatic vegetation recovery technology, which reconstructs good lake ecology in the destroyed lake environment; this new system constructs new vegetation that is significantly different from original vegetation. The third technology used in China is biological floating bed technology; it grows grains, vegetables, flowers and various green land plants in the water surface of eutrophication water. Song et al. (1998) treated the eutrophication water by rice cultivated by floating bed. Their results show that this treatment can efficiently reduce the total nitrogen and total phosphorus of the eutrophication water. Ma et al. (2000) with *Vetiveria zizanioides* cultivated by floating bed obtained similar results. These technologies contribute to the improvement of eutrophication of lakes and reservoirs. However, phytoremediation need a long restoration process, and is easily influenced by climate changes. Moreover, this technology is limited by short life cycles of the plants and generally small plant biomass.

CONCLUSIONS

Applications of plant biotechnology in China bring opportunities to meet the continuous increasing demands on food, energy and comfortable environment. In recent years, significant progresses were achieved in the development of biomass energy and the biocontrol of plant diseases and pests. The biofuels (bioethanol, biodiesel, activated carbon, biogas and biomass briquettes) are given priority to be achieved through the bioconversion of biomass residues, especially crop residues and forest residues. Crop straws/stovers, seed shells and food processing residues are successfully used for the preparation of biofuels. These applications diversify fuel supply and minimize the competitive utilization of plant resource for food purposes and environmental pollution. The applications of botanical pesticides (plant epiphytic yeasts) also reduce the negative effects of chemical fungicides on grains/edible plants and on the environment. However, research and development of botanical pesticide is slow. In addition, a higher price than chemical pesticide is unacceptable by most farmers.

Moreover, the applications of plant biotechnology in traditional Chinese medicine and phytoremediation of environmental pollution significantly fall behind the fields of biotech crops and biomass energy. For example, modern plant biotechnology has a narrow application in traditional Chinese medicine, particularly the identification of active constituents of TCM and the related action mechanisms. The selections of hyperaccumulator are mainly focused on the plants absorbing a few species (Cd, Zn, Cu, Mn, etc.). Also, phytoremediation of water eutrophication is limited by a long remediation period, short life cycles of the aquatic plants and generally small plant biomass, etc. The main directions for China's plant biotechnology are the conservation and utilization of abundant plant resources including wild crops, energy plants, medicinal plants, important flowers and hyperaccumulator plants, and the improvements of their attributes, as well as cultivation of new cultivars by the combination of traditional measures and modern plant biotechnology. Therefore, international cooperation and the cooperation between the enterprises, universities, and scientific research institutions should both be enhanced.

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