Site Specifics

Plant Molecular Biology in China: Opportunities and Challenges

HONGBO SHAO $^{1-3,*,+}$ and LIYE CHU³⁺

^{*I*} State Key Laboratory of Soil Erosion and Dryland Farming, Centre of Soil and *Water Conservation and Eco-environmental Research, Chinese Academy of Sciences and Northwest A&F Universit); Yangling 712100, China; 2Molecular Biology Laboratory, Bioinformatics College, Chongqing University of Posts & Telecommunications, Chongqing 400065,China; 3The Key Laborator)' of Biological Sciences, College of Molecular and Chemical Engineering, Qingdao University of Science and Technolog); Qingdao 266042, China*

Abstract. In the 21st century, mankind has witnessed great advances in life sciences, including completion of the *Arabidopsis thaliana* genome sequence and major advances with the rice genome. But, along with global economic development, urbanization, and depletion of natural resources, many serious problems are emerging (for example, environment, food, population, energy), which reinforce the need for sustainable development in many countries and in many institutions and prompt progress in life sciences globally. Plants offer the globe its only renewable resource of food, building material, and energy. Plants have highly sophisticated, concerted, short- and long-term adaptive mechanisms to the environment. Plants have great importance in global sustainable economic development. Plant molecular biology is a most essential and powerful tool in this process. Globally, plant molecular biology research is progressing rapidly, from use of model plants to cereal crops and from cellular processes to evolutionary mechanisms. Results of these studies have value in ecosystem regulation and environmental phytoremediation. China is a large agricultural country with one-fifth of the world's population. The gap for the level of plant molecular biology research in China is large, compared with that in other developed nations. However, some Chinese laboratories (notably those of academicians Jlayang Li, Zhihong Xu, Zhensheng Li, Mengmin Hong, Qifa Zhang and professors Yongbiao Xue, Shouyi Chen, and Zhen Zhu) have kept pace with international developments in plant molecular biology. How to fully utilize plant biodiversity in China requires future advances in plant molecular biology. This minireview discusses opportunities and challenges in plant molecular biology in China, analyzes the current status of international plant molecular biology, and provides suggestions to accelerate and advance international efforts.

^{*}These authors contributed to this paper equally.

[†]Author for correspondence, e-mail: shaohongbochu@hotmail.com;

shaohongbochu@ 126.com; ph: 029-87011190 and 0532-84022879.

Editorial note: This paper, with minor editing, is as it was presented by the authors at the Genetics Advancing Meeting of the Chinese Society of Genetics, Xiamen University, China, 13-17 May 2004.

Key words: Plant molecular biology, model plants, crops, biotechnology, microarray analysis, environmental stress

Introduction

International plant molecular biology has been progressing with great strides in the last decade, and especially in the last few years. This trend is reflected in influential international journals in plant sciences, for example, *Science, Nature, Cell, Proceedings of the National Academy of Sciences of the United States, Plant Cell, Plant Molecular Biology, Plant Physiology, Trends in Plant Science, Current Opinion in Plant Biology, Annual Review of Plant Physiology, Plant Molecular Biology, Plant, Cell and Environment, Plant Science, New Phytologist, Journal of Experimental Botany, Plant Journal, EMBO Journal, Molecular Biology and Evolution, Developmental Biology,* as well as some emerging journals, such as *Plant Biotechnology Journal, Comp Funct Genom, Electronic Journal of Biotechnology,* and *Bioinformatics.* Some Chinese journals also demonstrate this fact: *Acta Bot Sin, Chinese Bulletin of Sciences, Sciences in China, Prog Nat Sci, Acta Genetica Sin, Acta Ecol Sin, Journal of Plant Physiology and Molecular Biology, Cell Research,* and *Acta Phytoecologica Sin* (Shao et al., 2003a, 2003b). On the other hand, with large-scale urbanization and economic globalization, many severe problems have emerged since the 1980s, including food, energy, environment (global climate change), and water. People's quality of life depends on the availability of food, production of which requires an ideal ecoenvironment and sustainable utilization of natural resources. Plants offer the world its only renewable resource of food, building material, and energy, and they will play an important role in solving these problems (Bazzaz, 2001; Fiehn. 2002; Somerville and Dangl, 2000: Shao, 1993; Shao et al.. 2003a, 2004a). Plant molecular biology is a powerful tool for agricultural practices and other basic scientific and applied research activities (Vasil, 2003; Raghavendra et al., 2003). China, a large agricultural country with 20% of the earth's population, should make greater contributions to advance both international efforts in plant molecular biology and in sustainable development in China. This paper summarizes advantages of in plant molecular biology research in China and the opportunities and challenges it faces to prompt development and international cooperation in China.

Opportunities

Plant biodiversitv

China is abundant in plant biodiversity. Vegetation types range from forest, grassland, desert, and wetland in the north to tropical forest and tropical rain forest in the south. Various crops, vegetables, fruit trees, ornamental and medicinal plants have log history in cultivation. Favorable natural conditions, in particular for plants growing under extreme conditions, such as desert, aquatic, and stone plants, provide natural experimental systems for studying the molecular mechanism of plant responses to different environmental stresses (Jiang and Zhang, 2004; Shao et al., 2004a). Conditions that are important limiting factors influencing nlent orouth and development and crop production and quality include drought, salinity, cold, and acidity (Jain et al., 2001; Casati and Walbot, 2003; Chaves et al., 2003; Cushman and Bohnert, 2000; Lee et al., 2003; Editor's choice, 2003; Shinozaki et al., 2003; Sivamani et al., 2000; Tardieu, 2003; Shao et al., 2003a, 2003b).

Infrastructure

In the last 20 years, rapid developments in scientific infrastructure in China have taken place through establishment of State, Ministries of Education and Science and Technology, Provincial Key Laboratories and some laboratories pertaining to the Chinese Academy of Sciences. These laboratories provide a necessary platform to advance Chinese plant molecular biology, being well equipped with advanced instruments and facilities to study plant physiology, biochemistry, molecular biology, molecular genetics, crop improvement, and biotechnology. In addition, some laboratories are supported by Chinese institutions and foreign countries, such as the Sino-Max-Planck Laboratory in Shanghai.

Personnel resources

Chinese scientists have made recent advances in plant sciences research in agriculture, forestry, horticulture, medicine, and plant molecular biology in China. In addition, since 1995, many excellent younger Chinese scientists have returned to China, bringing strong scientific training and mastery of advanced molecular techniques. Some noteworthy examples are Professors Y. B. Xue, D. Luo, B. Han, Q. Liu, J. H. Zhang, L. J. Qiu, P. Wu, Kang Chong, J. S. Zhang, and Zanmin Hu. Many Chinese scientists who are leading academic experts have published influential papers in internationally famous journals, such as *Nature, Science, Plant Cell Proceedings of the National Academy of Sciences of the United States, Plant Physiology, Trends in Plant Biology, Plant Journal* and *EMBO Journal.* Many Chinese scholars are working, studying, or residing in foreign countries, for example, Professors X. W. Deng, H. Ma, D. Z. Zhao, J. K. Zhu, C. T. Lin, Z. B. Yang, P. X. Guo, and T. Zhu. These personnel resource serve China in several different ways to greatly elevate the level and accelerate progress of plant molecular biology in China.

Financial support

Along with development of Chinese economics, support from different ranks of government and regular and stable fund investment, Chinese plant molecular biology is stably advancing. The main programs and projects include the National Natural Science Foundation of China, the Knowledge Innovation Engineering of the Chinese Academy of Sciences, and plans from each province (autonomous region and centrally administrated city) and some ministries of China. In particular, grants supported by the Ministry of Science and Technology have been invested in the 863 Program (National Program of High Technology Development) and 973 Program (Key Basic Research Development Plan of China), of which plant molecular biology and biotechnology are major components. In addition, international funding channels have become more open to Chinese scientists. The main resources include the Rockefeller Foundation, McKnight Foundation, the Japan International Research Center for Agricultural Sciences (JIRCAS), the International

Foundation for Science, and many kinds of cooperative projects and contracts with the European Union, United States, Sweden, Japan, and other countries. These sources of financial support are essential in strengthening and supporting many excellent young scientists and actively enhancing the recognition of Chinese plant molecular biology.

Challenges

People of the current and future generations must have enough food to survive and prosper. Thus, a continuous food supply is always an important global focus, especially in developing countries. Population growth in China is estimated to reach 1.6 billion by the year 2030 and the demand for food production will increase by at least 60% to keep pace with that population growth. Globally, food production will need to be tripled to feed 12 billion human inhabitants by the year 2050 (Vasil, 2003; Vasil et al., 1992). This includes most of the people in China, as well as India, North Korea, and Thailand, whose dietary requirements are changing as a result of their improving buying power.

The second challenge is ecoenvironmental degradation, mainly deficits of water resources and pollution, soil erosion and desertification, decrease in biodiversity owing to widespread use of agrochemicals, and increase in natural disasters resulting from different forms of biotic and abiotic stressing factors (Vasil, 2003; Ballare, 2003; Liu, 2002; Rizhisky et al., 2002; Zhao et al., 2003; Zhu et al., 2003; T. Zhu, 2003; Shao et al., 2003a, 2004a, 2005a-2005e). These factors lead to great losses in food production annually. Plants are evolving (Abbott, 2003), and declining resources ensure a rapid decline in species diversity. With such severe global change and considering current technologies, biological measures (mainly plants) are the best to meet the food needs of the world's growing population and to protect and improve our ecoenvironment in a sustainable manner (Vasil, 2003; Shao et al., 2003a, 2003b). Plant molecular biology is the basis of plant biotechnology, a field that can solve the problem of increasing productivity on limited land under cultivation, with less water and under worsening ecoenvironmental conditions (Vasil, 2003; Somerville and Dangl, 2000; Miyao, 2003; Shao et al., 2004a; Vasil et al., 1992; Sopory and Maheshwari, 2004).

As a large agricultural country with 20% of the population of the earth, China's situation is more serious. With urbanization and emergence of peak population centers, Chinese ecoenvironmental crisis will be intensified. The central government has taken some measures in response to this situation, and some progress has been made in certain regions (Shao et al., 2003a, 2003b). The current generation can invest even more in research and education in plant molecular biology and plant biotechnology to benefit future generations (Bazzaz, 2001; Roberts, 2002).

Discussion

From the aspects reviewed above, it is clear that plant molecular biology and plant biotechnology are important technologies for increasing food production, improving food quality, and coping with ecoenvironmental problems. According

to Professor I. K. Vasil, of Florida University, internationally renowned plant biotechnologist and President of the 10th Congress of the International Association for Plant Tissue Culture & Biotechnology, "having considered all of the technologies known to us and the various arguments of the anti-biotechnology lobby, I remain more convinced than ever that plant biotechnology is still the best hope not only for meeting the food needs of the ever-growing human population, but also for conserving our precious but dwindling land and resources and preventing or even reversing environmental degradation" (Vasil, 2002a).

There are currently at least 8 trends in the area of international plant molecular biology: 1) extensive application of microarray techniques for the global analysis of gene expression in plants; 2) extension of technologies from model plants to crops; 3) transiting from manipulation in laboratories to natural fields (community, even ecosystem); 4) transiting from designing of individual factors to planning of multiple factors and their integration; 5) transiting from analyzing one gene at one developmental phase to dissecting many genes (hundreds, even thousands) during some stages or the whole life cycle simultaneously; 6) increasing use of the model organism public database, especially *Arabidopsis* and rice; 7) attention to the accurate link (metabolomics) between phenotypes and genotypes; and 8) extensive application of system-based methodology in plant molecular biology (Buell, 2002; Bais et al,, 2003; Eckardt, 2004; Editor's choice, 2003; Gesch et al., 2004; Lockhart and Winzeler, 2000; Koornneef and Stam, 2001; Pellegrineschi et al., 2002; Wisman and Ohlrogge, 2000; Saier, 1998; Tardieu, 2003; Rhee, 2000; Zhao et al., 2003; Fiehn, 2002; Sopory and Maheshwari, 2004).

These trends reflect the idea that application of new technology will provide deeper insights into the accurate molecular mechanisms of plant responses to their changing environment, under which plants, such as crops, grow and develop naturally and the collected experimental results are useful in practice (Wullschleger and Difazio, 2003; Singh, 1998; Rizhsky et al., 2002; J. K. Zhu, 2003; T. Zhu, 2003; Shao et al., 2004a; Vasil, 2002b). Some examples are presented below to illustrate these trends.

Cotton fibers are single-celled trichomes that differentiate from the epidermal layer of developing cotton ovules. They synchronously undergo a phase of rapid cell expansion, subsequently a stage of secondary cell wall deposition, and finally maturation. As a result, cotton fibers are unique in being one of the longest and most rapidly expanding plant cells and thus can provide a precious natural experimental system to study fundamental processes in plant molecular biology (Wullschleger and Difazio, 2003). The main elongation period of cotton fibers begins with the day of anthesis and is completed roughly 21days later, overlapping with the onset of secondary cell wall synthesis. Many genes involved in this period of development are transcriptionally regulated (Kim and Triplett, 2001). With the *Pilose* mutant, Arpart and Wilkins (2003) applied cDNA microarrays to analyze the underlying molecular mechanisms responsible for the decreased growth observed in *Pilose* fibers. Cotton cDNA arrays contained 4875 cDNAs, representing a unigene set of expressed sequence tags sequenced from isolated fibers 7-10 days after anthesis. Each probe was replicated 2 times on each slide. Cotton, *Arabidopsis,* and non-plant sequences were also spotted in replicate to offer a measure of nonspecific hybridization facilitated by poly-T tails of the cDNA probes. They found that 4 different targets representing α -expansins on the cotton cDNA array had 5-fold suppression in expression for fibers from the *Pilose* mutant. These data indicate that as part of a decreased cell growth rate, α -expansins are among the most strongly suppressed gene groups in elongating *Pilose* fibers. Arpart and Wilkins (2003) used the information from their microarrays to confirm whether the suppression of α -expansins is the primary and/or only determinant of decreased fiber length in the *Pilose* mutant. Their observation supports the view that the *Pilose* phenotype involves multiple mechanisms, including both expansins and cytoskeleton dynamics (Wullschleger and Difazio, 2003).

Similar research using maize has the broad goal of identifying the genes and biochemical networks that control the mechanisms of root growth and root-toshoot communication under drought, so as to further establish a deeper and more comprehensive understanding of the mechanisms that facilitate acquisition of water and mineral elements by roots, to elucidate the functions that roots play in adaptation to water-deficit conditions, and to transfer this knowledge to crop improvement through biotechnology. Sharp et al. (2003) recently demonstrated that maintenance of celt elongation in the apical zone of maize primary roots at low water potentials was associated with an increase in transcript levels for several expansin genes, as well as an increase in expansin activity and extractable expansin proteins (Al-ghazi et al., 2003; Cone et al., 2002; Chandler and Brendel, 2002). Sharp et al. (2003) have also shown that accumulation of ABA is required for maintenance of primary root growth of maize under water deficits. This result has been proved by other investigators (Casson and Lindsey, 2003; Chaves et al., 2003; Jain et al., 2001; Jiang and Zhang, 2004; Shinozaki and Dennis, 2003; Yu and Tang, 2004; Shao et al., 2004a, 2004b).

Transcript profiling can provide reliable data for identifying candidate genes underlying quantitative trait loci for yield and other agronomic traits in crops grown under a range of conditions (Liu, 2002). In addition, there is widespread interest in using DNA chips to monitor gene expression profiles for crops exposed to limited soil water availability (Schmidt, 2002; Riechmann et al., 2000). Many studies have shown that stress-inducible genes can be readily detected in plants exposed to fast dehydration (Cushman and Bohnert, 2000; Casson and Lindsey, 2003; Lee et al., 2003; Shinozaki et al., 2003; Sivamani et al., 2000; Xu et al., 1996). Seki et al. (2001) applied a larger microarray containing 1300 full-length cDNAs to identify 44 drought-inducing genes in *Arabidopsis* following a 2-h dehydration, whereas Talame et al. (2003) used the same technique to monitor large-scale changes and gene expression patterns in transcript abundance of barley *(Hordeum vulgare* L.) exposed to rapid (6-10 h) drought shock treatments. Their microarray analysis with mRNA extracted from water-shock and control tissues confirmed the changes in the expression level of transcripts, as reported by other researchers (Singh, 1998; Rizhsky et al., 2002; Xu et al., 1996). About 15% of all transcripts were either up- or down-regulated under conditions of rapid drought. Transcripts displaying significant up-regulation included jasmonate-responsible, metallothionein-like, and LEA- and ABA-responsive proteins. In the following experiment, water-stressed plants showed significant changes in transcript abundance after day 7 of water deficit, when the relative water content was $\sim 90\%$ -

93%.The greatest change in gene expression was observed after 11 days of treatment. The expression profile was analyzed by cluster analysis, and it was shown that the majority of treatment effects were due to transcript changes in the genes whose putative functions were involved in protein synthesis and turnover. The comparative analysis of the 2 sets of microarray data showed that the expression of a relatively large set of transcripts was differently regulated under water-shock or water-stress treatments. These results indicate that changes in gene expression vary considerably according to the dynamics of the water stress treatment (Wullschleger and Difazio, 2003; Shao et al., 2003a, 2003b, 2004a, 2004b). This also suggests that only a portion of the genes involved in intense and rapid dehydration may predict changes occurring when water stress develops in a slower fashion, which is more likely to happen under field conditions. Much evidence supports the view that a precise network-transcription regulation system controls plant responses to stress (J. K. Zhu, 2003; T. Zhu, 2003; Shinozaki and Dennis, 2003; Shao et al., 2003a, 2003b, 2004a, 2004b).

Aluminum is a major factor limiting crop productivity in acid soils, causing a large loss in yield for many plants. The main effect of aluminum toxicity is inhibition of root elongation. Different species or crop cultivars may respond to aluminum differently, and there is a molecular basis for this response. Some plants detoxify aluminum in the rhizosphere by releasing organic acids that chelate aluminum prior to root uptake, whereas others detoxify aluminum internally by forming complexes with organic acids. In attempt to better understand the molecular mechanisms associated with aluminum tolerance in wheat *(Triticum aestivum* L.), Guo et al. (2003) used cDNA microarrays to assess differences in gene expression for tolerant and susceptible cultivars exposed to aluminum. Seedlings of the cultivar Chisholm (aluminum-sensitive) and its near-isogenic line Chisholm-T (aluminum-tolerant) were grown in hydroponic culture. The aluminum tolerance of Chisholm-T was derived from the aluminum-tolerant cultivar Atlas 66. Threeday-old seedlings were exposed to a high, but sublethal, concentration of aluminum (10 mg/L); 24 h later, the seedlings were transferred to deionized water and rinsed. Aluminum-stressed and control roots were stained for 15 min with hematoxylin. High-intensity staining indicates accumulation of a high level of aluminum in roots. Light microscopy of roots 24 h after exposure to aluminum clearly showed that the roots of Atlas 66 and Chisholm-T were lightly stained, whereas the roots of the aluminum-sensitive cultivar Chisholm were very dark. Microarrays in this study were created by using suppression subtractive hybridization to identify transcripts that differed between cultivars after exposure to aluminum. This method was developed for generation of subtracted cDNA libraries and combines normalization and subtraction into a single procedure. The sequence tags from 1628 differently expressed cDNA clones were spotted on the array. Next, the arrays were hybridized with cDNA from the roots of Chisholm-T and Chisholm at 6 h, 1 day, 3 days, and 7 days after aluminum stress. Ninety-six differently expressed genes (64 unigenes) were identified during at least 1 of the 4 sampling periods. These genes were clustered into 6 groups according to their expression patterns; 3 groups were generally up-regulated and 3 groups were generally down-regulated. The genes showing strong differential expression between cultivars were sequenced, and putative functions were determined by homology

comparisons. The genes displaying up-regulation in the tolerant wheat cultivar at 1 or more time points included those coding for lipid transfer protein, jasmonateinducing protein, β -glucosidase, cellulose synthase peroxidase, acetylglutamate, kinase-like protein, and 2 disease resistance response proteins. Although most of the analyses for the results are exploratory, the results from this study should facilitate understanding of the pathways of expression and regulation of wheat genes under aluminum stress, provide insights into the genetic control of aluminum tolerance in wheat and other agricultural cereals, and open the door to ecoenvironmental improvement by phytoremediation and biotechnology (Wullschleger and Difazio, 2003; Chaves et al., 2003; Buell, 2002; Casati and Walbot, 2003; Shao et al., 2003a, 2003b).

The most important economic characteristics of agricultural grain crops are their yield, nutritional quality, and culinary characteristics. Yield and nutritional value are mostly determined by the synthesis and storage of carbohydrates, proteins, and minerals during grain filling, and culinary quality is affected by the interaction of various enzymes to produce the final structure of the starch at the molecular and granule levels. The manipulation of these pathways can result in significant improvements in nutritional value (Zhu et al., 2003; J. K. Zhu, 2003: Buell, 2002; Fiehn, 2002). For instance, reducing the amount of even one enzyme in the starch biosynthetic pathway (granule-bound starch synthase) can dramatically affect the culinary quality (Singh, 1998), resulting in softer, less sticky cooked rice. Many genes that participate in nutrient partitioning during grain filling and that affect starch quality have been identified. To understand how the expression of these genes is coordinated during grain development, a genomic approach to surveying the participation and interactions of all of the pathways is necessary. Using recently published rice genome information and referring to the *Arabidopsis* genome sequence (Buell, 2002; Han and Xue, 2003; Li et al., 2003; Riechmann et al., 2000; Wisman and Ohlrogge, 2000), Zhu et al. (2003) designed a rice GeneChip microarray that covers half of the rice genome. By monitoring the expression of 21,000 genes in parallel, they identified the genes involved in the grain filling process and found that the expression of genes participating in different pathways is coordinately controlled in a synchronized fashion during grain filling. They also found that an interesting, known promoter element in genes encoding seed storage proteins, AACA, was statistically over-represented among the 269 genes in different pathways with diverse functions that were significantly up-regulated during grain filling. By expression pattern analysis, a group of transcription factors that have the potential to interact with this element was identified. These investigators further found that most genes in the starch biosynthetic pathway exhibit multiple distinct spatial and temporal expression profiles, suggesting that different isoforms of a given enzyme are expressed in different tissues and at different developmental stages. Their results reveal key regulatory machinery and provide a relatively more accurate opportunity for modifying multiple pathways by manipulating key regulatory elements for comprehensive improvement of grain quality and quantity.

Species interaction is an important component for taxonomy, ecology, and evolutionary biology. Invasion by exotic plant species is a common mechanism for generation of plant biodiversity, but its molecular mechanism remains unknown,

which is also the core problem of allelopathy. Recently, Bais et al. (2003) used microarrays based on the *Arabidopsis* genome sequence database to identify the genes that are involved in secretion of toxic substances that kill the local plants. Their work truly constructs a bridge among ecology, evolutionary biology, and plant biology (Abbott, 2003; Soltis and Soltis, 2003; Schmidt, 2002). Ultraviolet radiation is an environmental stress factor. Casati and Walbot (2003) applied microarrays by referring to the *Arabidopsis* genome database to identify gene expression profiling in response to ultraviolet radiation in different maize genotypes with varying flavonoid content. Their results provide insights into the networkgene expression molecular mechanism of plant responses to biotic and abiotic stresses (Ballare, 2003; Shinozaki and Dennis, 2003; Shinozaki et al., 2003; Shao et al., 2003, 2004b, 2005a, 2005b, 2005d, 2005e; Wray et al., 2003; Girke et al., 2000).

The Future

Plants are open systems that need to survive and evolve during continuous adaptation to a changing environment. That task involves some things to retain from the past, some things to establish at present, and some things to strive for in the future, including transformation of matter, energy, morphology, and information. No doubt, all responses of plants to the surrounding environment should occur in order in a special-temporal regulated fashion, the molecular basis of which is concentrated in the transcription level of gene expression (Wray et al., 2003). Corresponding metabolic pathways to realize plant phenotypes interact coordinately and are controlled under such a network-regulating gene expression system (Fiehn, 2002; Shao et al., 2004a, 2005a-2005e; J. K. Zhu, 2003; T. Zhu, 2003). With the application of high-throughput, simultaneous microarray technology into different levels (molecular, subcellular, cellular, tissue, organ, individual, and population) of plants, comprehensive understanding of plant responses to environment will come soon in a post-genomic world, which will better instruct our sustainable development practice for better quality of life for humans.

Plant molecular biology is the basis of plant biotechnology, which is the best way to increase crop productivity. Since their emergence in 1983, more than 50 transgenic crops have been approved for commercial plantings, by 2003 more than 100 were undergoing field trials and/or regulatory review (Vasil, 2003), and these planting areas had expanded to >400 million acres to provide food for hundreds of millions of humans globally. Currently, transgenic crops are entering into the third generation with the coming of the genomic and post-genomic era (Vasil, 2003; Gesch et al., 2004; Miyao, 2003; Huang et al., 2002; Zhao et al., 2003; Shao et al., 2004a, 2005a, 2005d, 2005e; J. K. Zhu, 2003; T. Zhu, 2003; Pellegrineschi et al., 2002; Wray et al., 2003). Traits of third-generation transgenic crops include the following:

- 9 Genome sequencing/functional genomics/molecular breeding
- 9 Altered plant architecture
- Manipulation of flowering time, other circadian rhythms
- 9 Manipulation of fruit and seed quality, size, and number
- Improved photosynthetic efficiency
- 9 Improved nutrient assimilation
- Improved water use efficiency
- 9 Molecular phytoremediation
- 9 Exploiting and manipulating heterosis and apomixis
- 9 Exploiting transcription factor genes (complementation for quantitative characteristics in crops) for molecular breeding

In 1998, the US National Science Foundation (NSF) initiated Plant Genome Functions under Stresses; in 2000, NSF enhanced this project and made a plan for Plant Molecular Biology for 2003-2008 and 2010 (Editor's choice, 2003; Somerville and Dangl, 2000). In December, 2000, the *A. thaliana* genome sequence was completed; in September 2002, NSF initiated a maize genome sequencing program, one of the most important crops; in December 2002, the rice genome framework map was finished; recently, the complete sequence of the rice chromosome 8 centromere has been finished (Eckardt, 2004). Other crops, vegetables, or trees currently in different stages of sequencing are wheat, barley, soybean, potato, tomato, *Brassica, Lotus, Medicago,* poplar, and pine. This information, combined with functional genomics, will be of direct benefit to plant breeding. Consistency discovered in cereal genomes and other plants will be of significant value in the search for important genes. The discovery and identification of dwarfing and tillering genes in rice and others will be of much help in manipulating fruit and seed size and number, affecting productivity (Vasil, 2003; Li et al., 2003; Liu, 2002).

Facing such trends and considering the status of China. some suggestions may be beneficial to accelerating plant molecular biology and biotechnology in China.

- 9 Establish a national center for plant mutant cultivation and storage. Much plant physiological and biochemical work in China cannot be deepened and extended, mainly because there are no corresponding genetic mutants (lines) by which some work may be studied continuously at the molecular level.
- 9 Establish a regular training center of new technology for molecular biology. This is quite important in plant molecular biology and especially for young Chinese scholars. Mastering new knowledge and technology related to molecular biology is a key factor affecting development of plant molecular biology in China.
- 9 Popularize and intensify knowledge of bioinformatics, in particular utilization of public database and analysis of annotation.
- 9 Initiate national plant (crop) genome functions under stresses.
- Provide and guarantee financial support. Chinese governments at different ranks should invest more powerfully in this field to construct a solid platform for Chinese sustainable development and keep pace with the mainstream of the world.
- 9 Connect molecular biology data with ecology and evolutionary biology. The sciences of ecology and evolution will become more important as the issues that face humanity become more ecological in nature. This is also the reason that plant systems bioloby and competative phylogeny are emphasized to lay

• Reform the biology curricula in high schools and universities of China, which should emphasize the unity and systems of biology, especially at the molecular level. Plant biology students should have a basic understanding of molecular, cellular, and individual biology and ecosystem ecology.

The new techniques of molecular biology are constantly revolutionizing studies by plant biologists to explore the mechanisms of growth, development, maturation, aging, and the control of these processes to provide for human needs. This new era is in progress, and Chinese scientists should take full advantage of favorable conditions and grasp the opportunities and meet the challenges to stimulate the advance of plant molecular biology in China, which is our sacred responsibility and the task this great century confers on us.

Acknowledgements

Hongbo Shao is grateful to his adviser, Professor Shao Ming-an in the State Key Laboratory of Soil Erosion and Dryland Farming, Centre of Soil and Water Conservation and Eco-environmental Research, Chinese Academy of Sciences, for critical reading of the manuscript.

This study is jointly supported by 973 Program (G2000018605), Key Research Plan of NSFC (90102012), the Scientific and Technological Foundation of Chongqing University of Posts & Telecommunications (2005A-92), Doctoral Fund of Qingdao University of Science and Technology (QUST), and Shao Ming--an Innovation Group Project of Northwest A&F University.

This paper was presented at the Genetics Advancing Meeting of Chinese Society of Genetics, Xiamen University, Xiamen, China, 13-17 May 2004.

References

Abbott RJ (2003) Sex, sunflowers, and speciation. Science 301: 1189-1190.

- Al-ghazi Y, Muller B, Pinoche S, and Taylor, MN (2003) Temporal responses of *Arabidopsis* root architecture to phosphate starvation: evidence for the involvement of auxin signaling. Plant Cell Eviron 26: 1053-1066.
- Arpart AB and Wilkins TA (2003). Gene expression analysis of cotton *Pilose* mutant with altered fibre characteristics. Plant Animal Genome Conf XI, 2003 January 11-15, San Diego, CA, USA, pp 237-267.
- Bais HE Vepachedu R, Gilroy S, Callaway RM, and Vivanco JM (2003) Allelopathy and exotic plant invasion: from molecules and genes to species interaction. Science 301: 1377-1380.
- Ballare CL (2003) Stress under the sun: spotlight on ultraviolet-B responses. Plant Physiol 132: 1725-1727.
- Bazzaz FA (2001) Plant biology in the future. Proc Natl Acad Sci USA 98: 5441-5445.
- Buell CR (2002) Current status of the sequence of the rice genome and prospects for finishing the first monocot genome. Plant Physiol 130: 1585-1586.
- Casati P and Walbot V (2003) Gene expression profiling in response to ultraviolet radiation in maize genotypes with varying flavonoid content. Plant Physiol 132: 1768-1769.
- Casson SA and Lindsey K (2003) Genes and signaling in root development. New Phytol 158:11-34.
- Chandler VL and Brendel V (2002) The maize genome sequencing project. Plant Physiol 130: 1594-1597.
- Chaves MM, Maroco J, and Pereira J (2003) Understanding plant responses to drought-from genes to the whole plant. Funct Plant Biol 30: 239-264.
- Cone KC, McMullen MD, Bi IV, Davis GL, Yim YS, Gardiner JM, Polacco ML Sanchez-Villeda H, Fang Z, Schroeder SG, Havermann SA, Bowers JE, Paterson AH, Soderlund CA, Engler FW, Wing RA, and Coe EH Jr (2002) Genetic, physical, and informatics resources for maize. On the road to an integrated map. Plant Physiol 130: 1598-1605.
- Cushman JC and Bohnert HJ (2000) Genomic approaches to plant stress tolerance. Curt Opin Plant Biol 3: 117-124.
- Eckardt NA (2004) Journey to the center of the genome: complete sequence of the rice chromosome 8 centromere. Plant Cell 16: 789-791.
- Editor's choice (2003) The national plant genomics initiative: objectives for 2003-2008. Plant Physiol 130: 1741-1744.
- Fiehn O (2002) Metabolomics—the link between genotypes and phenotypes. Plant Mol Biol 48: 155-171.
- Gesch RW, Kang IH, Gallo-Meagher M, Gorte TH, and Frank MN (2004) Rubisco expression in rice leaves is related to genotypic variation of photosynthesis under elevated growth $CO₂$ and temperature. Plant Cell Environ 26: 32-43.
- Girke T, Todd J, Ruuska S, White J, Benning C, and Ohlrogge J (2000) Microarray analysis of developing *Arabidopsis* seeds. Plant Physiol 124: 1570-1581.
- Guo PG, Bai GH, Carver B, Wang DH, and Zhang LM (2003) Profiling expression of aluminum-tolerance genes in wheat through microarrays. Plant Animal Genome Conf XI, 2003 January 11-15, San Diego, CA, USA, pp 265-275.
- Han B and Xue YB (2003) Genome-wide intraspecific DNA-sequence variations in rice. Curr Opin Plant Biol 6: 134-138.
- Huang JK, Rozelle S, Pray C, and Wang QF (2002) Plant biotechnology in China. Science 295: 674-677.
- Jain AK, Basha SM, and Holbrook CC (2001) Identification of drought-responsive transcripts in peanut *(Arachis hypogaea* L.). Eur J Biol 4: 59-67.
- liang MY and Zhang JH (2004) Abscisic acid and antioxidant defense in plant cells. Acta Bot Sin 46: 1-9.
- Kim HJ and Triplett BA (2001) Cotton fibre growth in planta and in vitro. Models for plant cell elongation and cell wall biogenesis. Plant Physiol 127: 1361-1366.
- Koornneef M and Stam P (2001) Changing paradigms in plant breeding. Plant Physiol 125: 156-159.
- Lee JT, Prasad V, Yang PT, and John WK (2003) Transgenic tomato confers stress tolerance without affecting yield. Plant Cell Environ 26:1181-1190.
- Li X, Qian Q, Fu Z, Wang Y, Xiong G, Zeng D, Wang X, Liu X, Teng S, Hiroshi E Yuan M, Luo D, Han B, and Li J (2003) Control of tillering in rice. Nature 422: 618-621.
- Liu DJ (2002) Genome analysis in wheat breeding for disease resistance. Acta Bot Sin 44: 96-104.
- Lockhart DJ and Winzeler EA (2000) Genomics, gene expression, and DNA arrays. Nature 405: 827-836.
- Miyao M (2003) Molecular evolution and genetic engineering of C4 photosynthetic enzymes. J Exp Bot 54: 179-189.
- Pellegrineschi A, Ribaut JM, Trethowan R, White TJ, and Ribdon MN (2002) Progress in the genetic engineering of wheat for water-limited conditions. Japan International Research Center for Agricultural Sciences (JIRCAS) Working Rep pp 55-60.
- Raghavendra A, Vishnu Sane P, and Mohanty P (2003) Photosynthesis research in India: transition from yield physiology to molecular biology. Photosynthesis Res 76: 435- 450.
- Rhee SY (2000) Bioinformatic resources, challenges, and opportunities using *Arabidopsis* as a model organism in a post-genomic era. Plant Physlol 124: 1460-1464.
- Riechmann JL, Heard J, Martin G, Riechmann JL, Heard J, Martin G, Reuber L, Jiang C, Keddie J, Adam L, Pineda O, Ratcliffe OJ, Samaha RR, Creelman R, Pilgrim M, Broun E Zhang JZ, Ghandehari D, Sherman BK, and Yu G (2000) *Arabidopsis* transcription factors: genome-wide comparative analysis among eukaryotes. Science 290: 2105-2109.
- Rizhisky L, Liang HJ, and Mittler R (2002) The combined effect of drought stress and heat shock on gene expression in tobacco. Plant Physiol 130: 1143-1157.
- Roberts JKM (2002) Proteomics and a future generation of plant molecular biologists. Plant Mol Biol 48: 143-154.
- Saier MH Jr. (1998) Genome sequencing and informatics: new tools for biochemical discoveries. Plant Physiol 117:1129-1133.
- Schmidt R (2002) Plant genome evolution: lessons from comparative genomics at the DNA level. Plant Mol Biol 48: 21-37.
- Seki M, Narusaka M, Abe H, Kasuga M, Yamaguchi-Shinozaki K, Carninci R Hayashizaki Y, and Shinozaki K (2001) Monitoring the expression pattern of 1300 *Arabidopsis* genes under drought and cold stresses by using full-length cDNA microarray. Plant Cell 13: 61-72.
- Shao HB (1993) The Developing Frontier and Perspective of Molecular Biology, 1993, pp 88-116, Agricultural Sci-tech Press, Beijing.
- Shao HB, Liang ZS, and Shao MA (2003a) Roles of ABA during seed development and maturation of higher plants. For Studies China 5: 35-44.
- Shao HB, Liang ZS, and Shao MA (2003b) Molecular biology and biotechnological measures for improving the ecoenvironment of Loess Plateau in China. Trans CSAE 19: 19-24.
- Shao HB, Liang ZS, and Shao MA (2004a) Impacts of transgenic crops on ecoenvironment and their trend. Trans CSAE 20: 42-51.
- Shao HB, Liang ZS, Shao MA, and Wang BC (2004b) Changes of some physiological and biochemical indices for soil water deficits among 10 wheat genotypes at seedling stage, Colloids Surfaces B: Biointerfaces 42: 107-113.
- Shao HB, Liang ZS, Shao MA, and Wang BC (2005a) Investigation on the physiological and molecular mechanism of effects from PEG-6000 during the in vitro responses of barley mature embryos. Colloids Surfaces B: Biointerfaces 41: 72-77.
- Shao HB, Liang ZS, and Shao MA (2005b) Changes of some anti-oxidative enzymes under soil water deficits among 10 wheat genotypes at tillering stage, J Sci Food Agric 86 (in press).
- Shao HB, Liang ZS, and Shao MA (2005c) LEA proteins in higher plants: structure, functions, and gene regulation. Colloids Surfaces B: Biointerfaces 45: 131-135.
- Shao HB, Liang ZS, and Shao MA (2005d) Dynamic changes of anti-oxidative enzymes of 10 wheat genotypes through life circle at soil water deficits. Colloids Surfaces B: Biointerfaces 42: 187-195.
- Shao HB, Liang ZS, and Shao MA (2005e) Adaptation of higher plants to stress environment and stress signal transduction. Acta Ecol Sin 25: 1772-1781.
- Sharp RE, Bohnert HJ, Davis GL, Wu TP, and Groodman MN (2003) Root growth maintenance during water deficits: physiology to functional genomics. Plant Animal Genome Conf XI, 2003 January 11-15, San Diego, CA, USA, pp 5-12.
- Shinozaki K and Dennis ES (2003) Cell signaling and gene regulation global analysis of signal transduction and gene expression profiles. Curr Opin Plant Biol 6: 405-409.
- Shinozaki K, Yamaguchi-Shinozaki K, and Seki M (2003) Regulatory network of gene expression in the drought and cold stress responses. Curr Opin Plant Biol 6: 410-417.
- Singh KB (1998) Transcriptional regulation in plants: the importance of combinatorial control. Plant Physiol 118:1111-1120.
- Sivamani E, Bahieldinl A, Wraith JM, A1-Niemi T, Dyer WE, Ho TD, and Qu R (2000) Improved biomass productivity and water use efficiency under water deficit conditions in transgenic wheat constitutively expressing the barley HVAY 1 gene. Plant Sci 156: 227-233.
- Soltis DE and Soltis PS (2003) The role of phylogenetics in comparative genetics. Plant Physiol 132: 1790-1800.
- Somerville C and Dangl J (2000) Plant biology in 2010. Science 290: 2077-2078.
- Sopory SK and Maheshwari SC (2004) Plant molecular biology in India--the beginnings. Curr Sci 80: 270-279.
- Talame V, Ozturk N, Bohnert HJ, Moris LN, and Charles PN (2003) Microarray analysis of transcript abundance in barley under conditions of water deficit, Plant Animal Gehome Conf XI, 2003 January 11-15, San Diego, CA, USA, pp 15-24.
- Tardieu F (2003) Virtual plants: modeling as a tool for the genomics of tolerance to water deficit. Trends Plant Sci 8: 9-14.
- Vasil IK (2002a) Presidential address to the 10th Congress of the International Association for Plant Tissue Culture and Biotechnology (IAPTC&B), 2002 June 23-28, Florida, CA, USA, pp 1-18.
- Vasil IK (2002b) Turning point articles: the wanderings of a botanist. In Vitro Cell Dev Biol-Plant 38: 383-395.
- Vasil IK (2003) The science and politics of plant biotechnology—a personal perspective. Nat Biotechnol 21: 849-851.
- Vasil V, Castillo AM, Fromm ME, and Vasil IK (1992) Herbicide resistance fertile transgenic wheat plants obtained by microprojectile bombardment of regenerable embryogenic calli. Bio/Technology 10: 667-674.
- Wisman E and Ohlrogge J (2000) *Arabidopsis* microarray service facilities. Plant Physiol 124: 1468-1471.
- Wray GA, Hahn MW, Abouheif E, Balhoff JP, Pizer M, Rockman MV, and Romano LA (2003) The evolution of transcriptional regulation in eukaryotes. Mol Biol Evol 20: 1377-1419.
- Wullschleger SD and Difazio SP (2003) Emerging use of gene expression microarrays in plant physiology. Comp Funct Genom 4: 216-224.
- Xu D, Duan X, Wang B, Hong B, Ho T, and Wu R (1996) Expression of a late embryogenesis abundant protein gene, HVA 1, from barley confers tolerance to water deficit and salt stress in transgenic rice. Plant Physiol 110: 249-257.
- Yu SW and Tang KX (2004) MAP kinase cascades responding to environmental stress in plants. Acta Bot Sin 46: 127-136.
- Zhao FY, Guo SL,Wang ZL, Wang GH, Zhang H, and Tian H (2003) Recent advances in study on transgenic plants for salt tolerance. J Plant Physiol Mol Biol 29: 171-178.
- Zhu JK (2003) Regulation of ion homeostasis under salt stress. Curr Opin Plant Biol 6: 441-445.
- Zhu T (2003) Global analysis of gene expression using GeneChip microarrays. Curr Opin Plant Biol 6: 418-425.
- Zhu T, Budworth P, Chen WQ, Zhang WH, and White TP (2003) Transcriptional control of nutrient partitioning during rice grain filling. Plant Biotech J 1: 59-70.