

## Trends in Plant Molecular Biology

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### 3.1 Introduction

The principal goal of plant molecular biology is to reveal the mechanisms for controlling forms and metabolism, and the evolutionary process of plants with focus on the gene function. This field of study is highly expected to boost development of plants necessary for solving food, environmental and energy problems in the future. In addition, plant molecular biology is recognized as a crucial field of study in the Comprehensive Strategy to Promote Science and Technology, which was adopted at the Council for Science and Technology Policy of the Cabinet Office in September 2001.

Studies on the gene function of so-called model plants such as *Arabidopsis thaliana* and rice have

progressed all over the world since the mid-1980s for several reasons. First, the fruits of genetic and physiological studies on these plants have been accumulated for a long time. Besides, their genome sizes are small, mating and gene transfer are uncomplicated, and biological resources for gene function analysis can be easily obtained.

A joint project of Japan, the U.S. and Europe completed genome sequence in *Arabidopsis* in December 2000 for the first time in higher plants. Meanwhile, Syngenta of Switzerland and the Beijing Genomics Institute of China separately completed genome sequence in rice in April 2002. Moreover, an international consortium, which Japan takes a leading part, is expected to complete more accurate genome sequence in rice by the end of 2002. The research basis for plant gene function analysis is solidifying, as whole genome

**Table 1:** Number of sequenced plant nucleotides registered in the DDBJ/EMBL/GenBank DNA databases (as of April 2002).

Ranking	Scientific name (Common name)	Number of registered nucleotides (b) *1	Genome size (Mb) *2
1	<i>Oryza sativa</i> (Rice)	397,636,312	430
2	<i>Arabidopsis thaliana</i> (Arabidopsis)	313,816,117	125
3	<i>Brassica oleracea</i> (Cabbage, broccoli)	195,244,865	1,200
4	<i>Glycine max</i> (Soybean)	116,211,613	1,290~1,810
5	<i>Zea mays</i> (Maize)	102,365,381	2,300
6	<i>Lycopersicon esculentum</i> (Tomato)	84,099,550	950
7	<i>Medicago truncatula</i> (Barrel medic)	73,695,194	450
8	<i>Hordeum vulgare</i> (Barley)	70,306,697	4,800
9	<i>Chlamydomonas reinhardtii</i> (Chlamydomonas)	64,781,512	100
10	<i>Sorghum bicolor</i> (Sorghum)	42,412,607	750
11	<i>Triticum aestivum</i> (Wheat)	37,072,790	16,000
12	<i>Solanum tuberosum</i> (Potato)	36,961,099	—
13	<i>Physcomitrella patens</i> (Moss)	25,834,542	400
14	<i>Pinus taeda</i> (Pine)	18,645,322	—
15	<i>Lotus japonicus</i> (Lotus)	17,707,239	440~490

\*1 Registered nucleotides include not only genomes but also cDNAs.\*1

\*2 One Mb is equal to  $1 \times 10^6$  b.

Source: Authors' compilation on the basis of DDBJ's statistical data

sequences of rice and *Arabidopsis* are revealed.

In the meantime, international competition in gene function analysis is becoming keener as firm research bases like whole genome sequence information and biological resources are established. Therefore, also in Japan, we immediately need to obtain study results that will contribute to solving food, environmental and energy problems.

In this report, we will survey trends in plant molecular biology in Japan and other countries, and discuss about the policy for promoting this field of study in our country.

## 3.2 History of plant molecular biology

### 3.2.1 Plant species with advanced gene analyses

Table 1 shows the amount of each plant's sequenced nucleotides registered in the DDBJ/EMBL/GenBank DNA databases. This list indicates what kinds of plants have been mainly studied in plant molecular biology.

When the amount of sequenced nucleotides registered in this database is compared among plant species, rice has the largest number of sequenced nucleotides. Rice has been studied as a model plant of Poaceae, which includes various agricultural plants such as rice, maize and wheat. Meanwhile, *Arabidopsis thaliana* takes second place. *Arabidopsis* is a model plant of higher plants and its worldwide gene function analysis has been promoted since the mid-1980s. The amount of sequenced nucleotides of these two model plants surpasses that of other plants. Researchers all over the world are pursuing the

study of these two species as major materials for unveiling the gene function.

In third place comes cabbage and broccoli, which belongs to Brassicaceae, the same family as *Arabidopsis*. Researchers have promoted the study of the gene function of cabbage and broccoli, making good use of its gene homology with *Arabidopsis*.

Soybean takes fourth place. Soybeans are cultivated around the world as a source of protein and oil, and nitrogen fixation by their symbiotic bacteria is notable. Meanwhile, *Medicago truncatula* and *Lotus japonicus*, model plants of Fabaceae, take the seventh and 15th places, respectively.

### 3.2.2 *Arabidopsis thaliana*

Table 2 shows the chronology of research on *Arabidopsis*.

*Arabidopsis* is a wildflower distributed around the Northern Hemisphere. Basic research on *Arabidopsis* as a material for genetics began in Germany around 1965. Later, *Arabidopsis* came to be widely used as a major material for plant molecular biology due to its advantages. For example, though its genome size is as small as 125Mb, it has basic gene functions that a higher plant has, such as growth, flowering, environmental response, and pest resistance. In addition, it has a rapid life cycle of about two months. Furthermore, its gene manipulation is relatively easy.

Researchers in Japan, the US and Europe established an international research organization to study *Arabidopsis* in 1990. This grew into a whole genome sequencing project in 1995. Then, genome sequence of *Arabidopsis* was completed

**Table 2:** Chronology of *Arabidopsis* research

1965	Basic research such as isolation of mutants is conducted in Germany.
1985	Extensive application of molecular genetics starts in the U.S., Europe, Japan, Australia and other countries.
1990	A committee for promoting international joint research of <i>Arabidopsis</i> is established. Gene introduction becomes generalized, and production of tag lines*2 and gene cloning begin.
1995	A whole genome sequencing project is launched with international cooperation.
2000	Genome sequence is completed. The 2010 Project is initiated. Analysis of the function and interactive network of the gene starts. Research on mechanisms of diversification and evolution by comparison with genomes of various plants begins.

Source: the figure made by Prof. Kiyotaka Okada of the Graduate School of Science, Kyoto University

in December 2000. The Kazusa DNA Research Institute, which is subsidized by Chiba Prefecture, was the Japanese organization that joined this international project. This institute decoded 30% of the entire genome, the largest amount among the six groups joined in the project, and its achievement is recognized throughout the world.

### 3.2.3 Rice

Rice, as well as being an agricultural plant, serves as a model plant in clarifying the common gene function of Poaceae like maize and wheat. Japan launched a rice-genome-project in 1991, the first in the world on this subject, and established the basis for rice genome studies by mass analysis of cDNAs<sup>\*1</sup> and construction of high-density gene linkage maps<sup>\*3</sup> and chromosome maps.

In 1998, the International Rice Genome Sequencing Project (IRGSP) was established as the second rice-genome-project with Japan as its leading country, and started whole genome sequencing of rice. As of May 2002, 317Mb or 74% of the entire rice genome (430Mb) has been sequenced, and the National Institute of Agrobiological Sciences and the Society for Techno-innovation of Agriculture, Forestry and Fisheries have jointly sequenced about 60% of the nucleotides. This project will complete high-accuracy sequencing of the major parts of rice genome by the end of 2002.

In the meantime, Syngenta of Switzerland (a multinational enterprise ranking first in the field of agricultural chemical and third in the high-value commercial seeds market of the world) and the Beijing Genomics Institute of China separately completed draft genome sequence in rice in April 2002 (*Science*, April 5, 2002).

Some Japanese and foreign researchers using rice had said, "IRGSP must immediately release nucleotide sequence information covering the entire genome even if its accuracy is low, so that anyone can use it." To meet such a request, IRGSP, in 2001, changed its policy of unveiling data after nucleotides were completely sequenced at an accuracy of 99.99%: It decided to release data earlier even if some regions are not sequenced.

Meanwhile, international consensus exists on the need for whole genome sequencing in rice at an

accuracy of 99.99%, or with less than one nucleotide sequencing error in 10,000 base pairs (bp). For example, Syngenta, which already completed draft genome sequence in rice, agreed to offer its data for free to IRGSP in order to help with the completion of the high-accuracy sequencing. In the meantime, according to a press release on May 6, 2002 by the Rockefeller Foundation, which has subsidized plant biological researches to solve food and environmental problems, Dr. Gordon Conway, president of the foundation, praised Japan for its initiative in rice genome sequencing. He stated, "Japan's leadership and commitment to accurately sequencing the rice genome will enable research leading to better food security throughout the developing world." Moreover, he urged that this project "be carried to completion."

### 3.2.4 Fabaceae

In world food production, monocots such as rice, wheat and maize are cultivated as starch sources, and fabaceous plants such as soybeans are grown as protein sources. Fabaceae is an important object of study in plant molecular biology, because it has marked features such as protein accumulation in the seed and nitrogen fixation by bacterium living symbiotically in their roots.

Gene function analysis is progressing around the world with intensive studies on two model plants, *Medicago truncatula*, a relative of alfalfa, and *Lotus japonicus*, an indigenous species in Japan.

The US and France heavily subsidize research on *Medicago*, and gene mapping, preparation of tag lines<sup>\*2</sup> and symbiotic-bacteria-genome sequencing are more advanced on this plant than in *Lotus*. European and U.S. research groups are taking the initiative in *Medicago* studies.

In Japan, analysis of expressed sequence tags (EST)<sup>\*4</sup> of *Lotus* is progressing with contributions by the Kazusa DNA Research Institute, because *Lotus* is Japan's indigenous species and its genetically diverse strains are stocked. In the meantime, this institute, in December 2000, completed genome sequence in *Mesorhizobium loti*, a symbiotic bacteria with a genome size of 7.6 Mb, which lives symbiotically in *Lotus* and contributes to nitrogen fixation.

### 3.3 Recent progress in plant molecular biology

#### 3.3.1 Estimate of the gene function

In *Arabidopsis* and rice, with their whole genomes sequenced, the total number of genes in their genomes and gene functions can be estimated from the genome sequence information on the basis of homology with genes with unveiled functions (Figure 1).

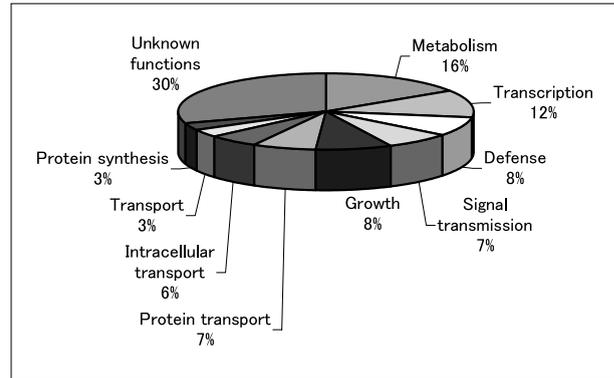
From homology with nucleotide sequences of particular genes that are already analyzed, the *Arabidopsis* genome presumably contains approximately 25,500 genes. It has been revealed that a considerable part of the genes are related to metabolism and gene expression control. Yet, the functions of about 30% of all the genes could not be assessed at the time of genome-sequence completion.

#### 3.3.2 Genes related to morphogenesis

The whole genome sequence of *Arabidopsis* has been revealed and gene function analysis has proceeded. Among various functions of plant genes, several features of genes involved in morphogenesis, or genes determining forms, have been clarified:

- 1) Functional change of a few genes brings about remarkable change in forms.
- 2) Functional change of genes expressing in the apical meristem of the stem brings about remarkable change in the plant form.
- 3) Genes related to intercellular transmission of the positional information play an important role in cell proliferation and differentiation.

**Figure 1:** Functions of about 25,500 genes that *Arabidopsis* genome presumably contains



Source: the figure made by Prof. Kiyotaka Okada of the Graduate School of Science, Kyoto University

- 4) More than one gene has the same function in many cases.

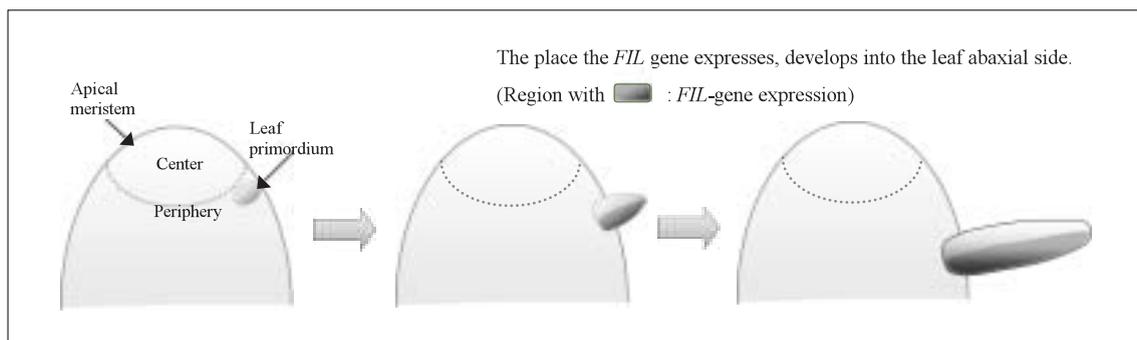
Genes involved in plant morphogenesis are important objects of basic research because they often affect such traits as yield and quality of commercial plants like crops.

#### 3.3.3 Genes to determine abaxial and adaxial sides of the leaf

Cell division is active in the apical meristem of the plant stem, and the primordium, or the tissue that develops into plant organs such as the leaf and flower, is generated there. Positions of leaf primordium cells relative to the meristem probably determine the abaxial and adaxial sides of the primordium. Different genes are presumably expressing in the abaxial and adaxial sides because their structures in a mature leaf are distinct.

The research team of Kiyotaka Okada, professor of the Graduate School of Science, Kyoto University, isolated the *FILAMENTOUS FLOWER*

**Figure 2:** Expression process of the *FIL* gene



Source: the figure made by Prof. Kiyotaka Okada of the Graduate School of Science, Kyoto University

(*FIL*) gene, making good use of a mutant that cannot successfully develop leaf abaxial and adaxial sides of *Arabidopsis* (Figure 2). The team discovered that the place the *FIL* gene expresses, develops into the leaf abaxial side. Moreover, detailed structural analysis of the *FIL* gene revealed that the region of 50 bp from 1745 bp to 1795 bp upstream of the start codon of the *FIL* gene has a promoter of the *FIL* gene, so that the *FIL* gene expresses in the leaf abaxial side.

Today, the gene involved in the control of *FIL*-gene expression is about to be isolated. This gene presumably encodes the protein binding to the above-mentioned region of 50 bp. That is, researchers are trying to identify the signal serving as the positional information, supposing *FIL*-gene expression is controlled as follows. First, this signal is transmitted from the center of the meristem to the leaf primordium. Then, the strength of the signal varies in the abaxial and adaxial sides of the primordium due to the difference in their distance from the meristem, thereby affecting *FIL*-gene expression.

It has been understood that various signal transmissions are related not only to the leaf abaxial-adaxial determination but also to other important stages of plant morphogenesis such as flower bud differentiation and fertilization. Almost all plants including crops probably have a common mechanism of signal transmission, so clarification of this mechanism with such model plants as *Arabidopsis* is crucial in plant molecular biology.

### 3.3.4 Genes related to the height of rice

The International Rice Research Institute (IRRI) of the Philippines selected the high-yielding variety IR8 through breeding in the 1960s. IR8, being short and hardly lodging, greatly contributed to the increase in food production in Asian countries, which is known as the Green Revolution.

The research group of Makoto Matsuoka, professor of the BioScience Center, Nagoya University, isolated the *sd1* gene, which was deeply related to the Green Revolution as a gene involved in rice-height determination (Nature, April 18, 2002).

The *sd1* gene, which encodes the GA20<sub>ox</sub>-2

**Figure 3:** Mutation in the *sd1* gene, which is involved in gibberellin synthesis, makes the plant height short: left, wild-type rice; right, *sd1*-mutant rice.



Source: the home page of the BioScience Center, Nagoya University

enzyme related to biosynthesis of gibberellin, a plant hormone, is deleted in IR8. Plant height decreases in this variety because of the reduction of gibberellin (Figure 3).

Mutation of the isolated *sd1* gene can realize an ideal height of rice, so molecular breeding using this gene may become possible in other rice varieties as well.

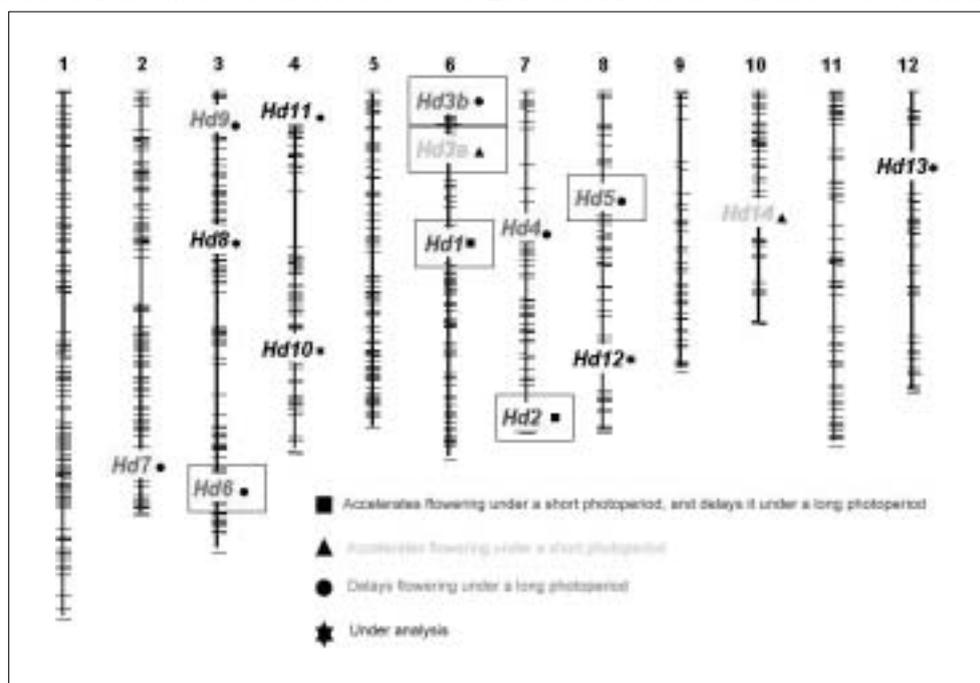
### 3.3.5 Genes controlling the flowering time

Most quantitative traits such as flowering time have been hardly researched: Various genes control them, so that analysis of such traits has been complex. However, molecular genetic analysis of the characters determined by quantitative trait loci (QTL) is becoming feasible with DNA markers<sup>\*5</sup> and nucleotide-sequence information obtained through rice genome studies.

The research team led by Masahiro Yano of the Department of Molecular Genetics, National Institute of Agrobiological Sciences, generated a population for gene analysis derived from a cross between rice varieties Nipponbare and Kasalath by such hybridization methods as replacement of certain chromosomes. Based on gene analyses with DNA markers, this group found 15 QTL regions in chromosomes related to the rice flowering time (Figure 4).

Moreover, this team isolated genes (*Hd1*, *Hd3a* and *Hd6*) related to the flowering time from three QTL regions by map-based cloning<sup>\*6</sup> and analyzed their gene structures. It revealed that the *Hd1* and *Hd3a* genes are homologues with the flowering-related genes of *Arabidopsis*, while the *Hd6* gene

**Figure 4:** Locations in chromosomes and functions of QTLs involved in the determination of the flowering time of rice.



\*Frames indicate genes involved in photopathology among the QTLs detected.

Source: a figure made at the National Institute of Agrobiological Sciences

is homologues with the biological-clock-associated genes of *Drosophila* and *Arabidopsis*. These results indicated that rice, a short-day plant (a plant that flowers under a short photoperiod), and *Arabidopsis*, a long-day plant, have flowering-related genes with similar structures, although they have a converse response to the day length (*Plant Cell*, December 2000; *Proceedings of the National Academy of Sciences*, July 3, 2001).

The method of analyzing rice QTLs with DNA markers, which Yano's research group established, can be applied to analysis of traits other than the flowering time. Thus, this technique will help researchers to understand the control mechanism of genes related to useful traits, whose analysis have been difficult.

### 3.3.6 Genes related to resistance to environmental stress

Development of technology to improve plant tolerance to environmental stresses such as high and low temperatures, drought and salt has been hoped for to solve food and environmental problems. However, only recently have such research and development activities proceeded because the resistance mechanism is complicated. Lately, researchers have isolated genes that are induced by drought and salt stresses and encode proteins involved in acquisition of stress

resistance, as well as many transcription factors\*<sup>7</sup> controlling the expression of these genes.

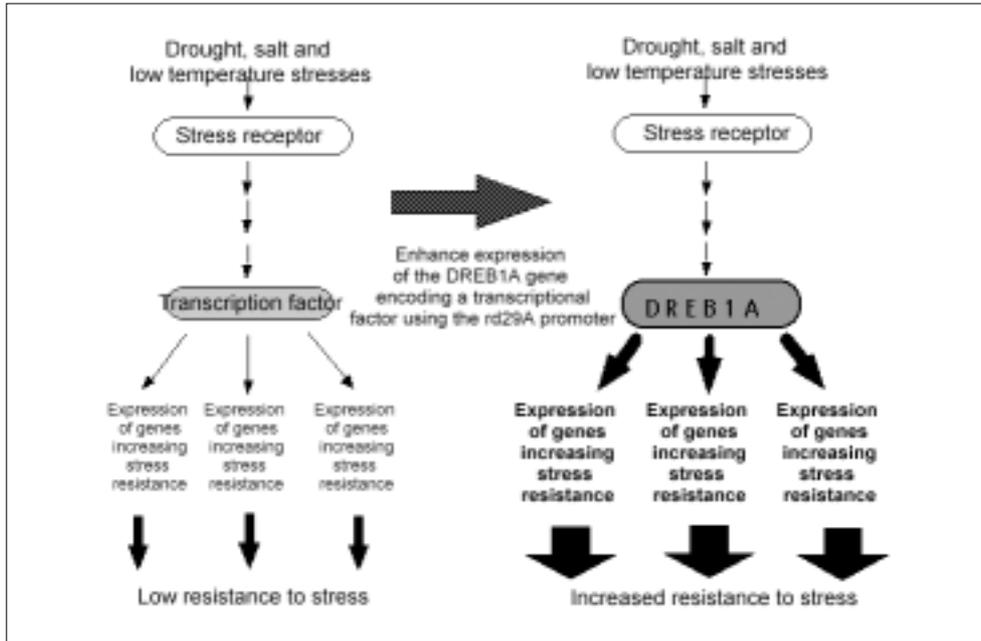
The research group of Kazuko Shinozaki, a chief researcher at the Japanese International Research Center for Agricultural Sciences, isolated the *DREB1A* gene that encodes the transcription factor controlling gene expression in response to drought, salt and low temperature by analyzing various genes related to the resistance mechanism of *Arabidopsis*. In addition, when the rd29::DREB1A construct, consisting of the *DREB1A* transgene and the rd29A promoter enhancing transgene expression under stress, was introduced into *Arabidopsis*, this transgenic plant showed high tolerance to drought, salt and frost (Figure 5).

The environmental-response mechanism controlled by the transcription factor encoded by the *DREB1A* gene is probably common to plants. Thus, it is greatly hoped that combination of the *DREB1A* gene and the rd29A promoter will be applied to the development of environmental-stress-resistant varieties of trees and crops like rice, wheat and maize.

## 3.4 2010 Project in progress

In 2000, when the *Arabidopsis* genome sequence was completed, Japanese, US and

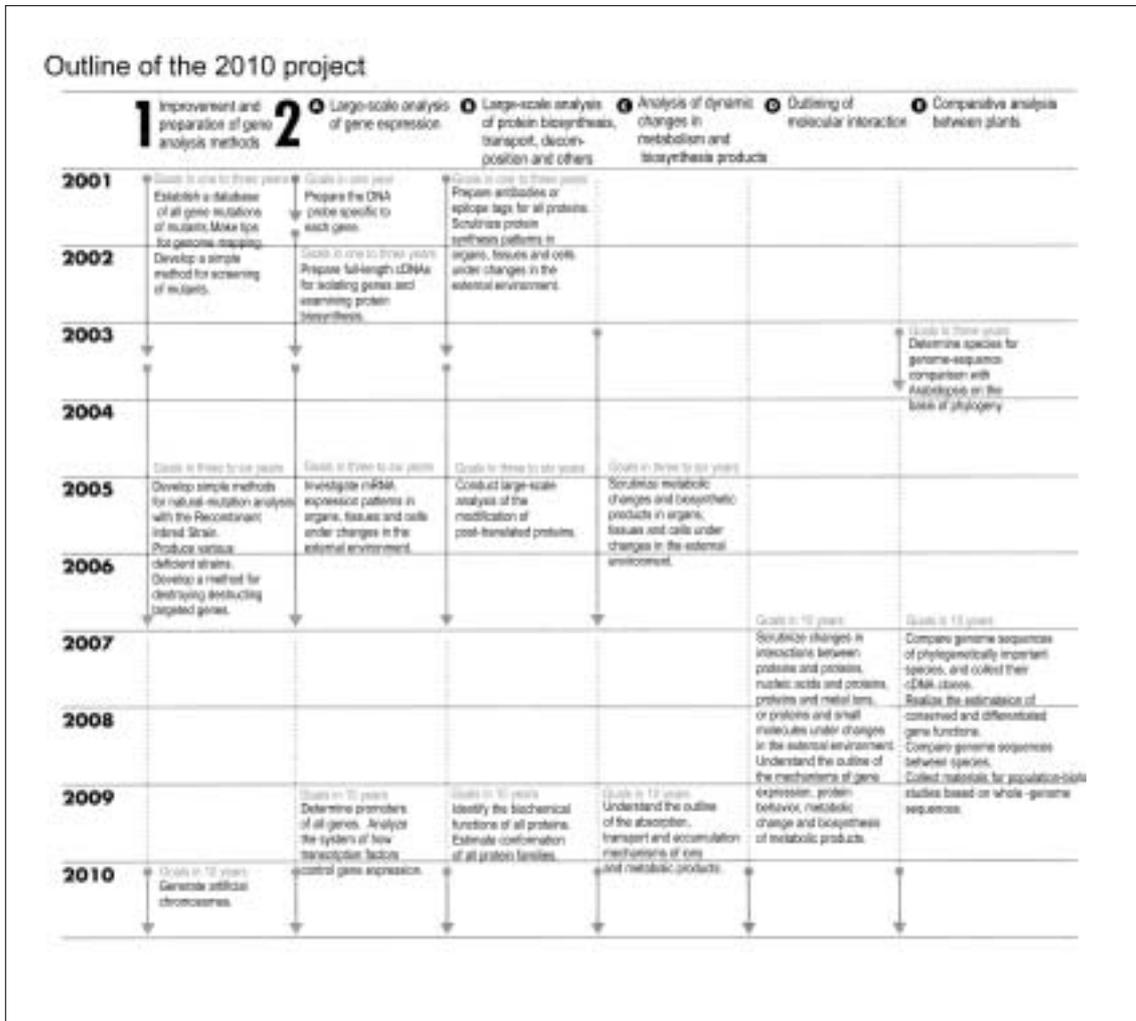
Figure 5: Mechanism of increasing resistance to environmental stress



\*Though three stress-resistance-increasing genes expressed by DREB1A-gene expression are drawn in this chart for illustrative purpose, various genes are actually expressed thereby increasing stress tolerance.

Source: a figure made at the Japan International Research Center for Agricultural Sciences

Figure 6: Outline of the 2010 project



Source: the figure made by Prof. Kiyotaka Okada of the Graduate School of Science, Kyoto University

European researchers of plant molecular biology jointly drew up and announced the 2010 Project with the support of the National Science Foundation of the US (Figure 6).

In this program, researchers from Japan, the U.S., Europe and other countries are conducting detailed function analysis of each gene. This project plans to comprehensively analyze the genes, proteins and metabolism of mainly *Arabidopsis* within the decade by 2010. Such studies using model plants will broaden our understanding of higher plants at the molecular level.

### 3.5 Hurdles in promoting plant molecular biology

#### 3.5.1 Plant molecular biology and large-scale sequencing projects

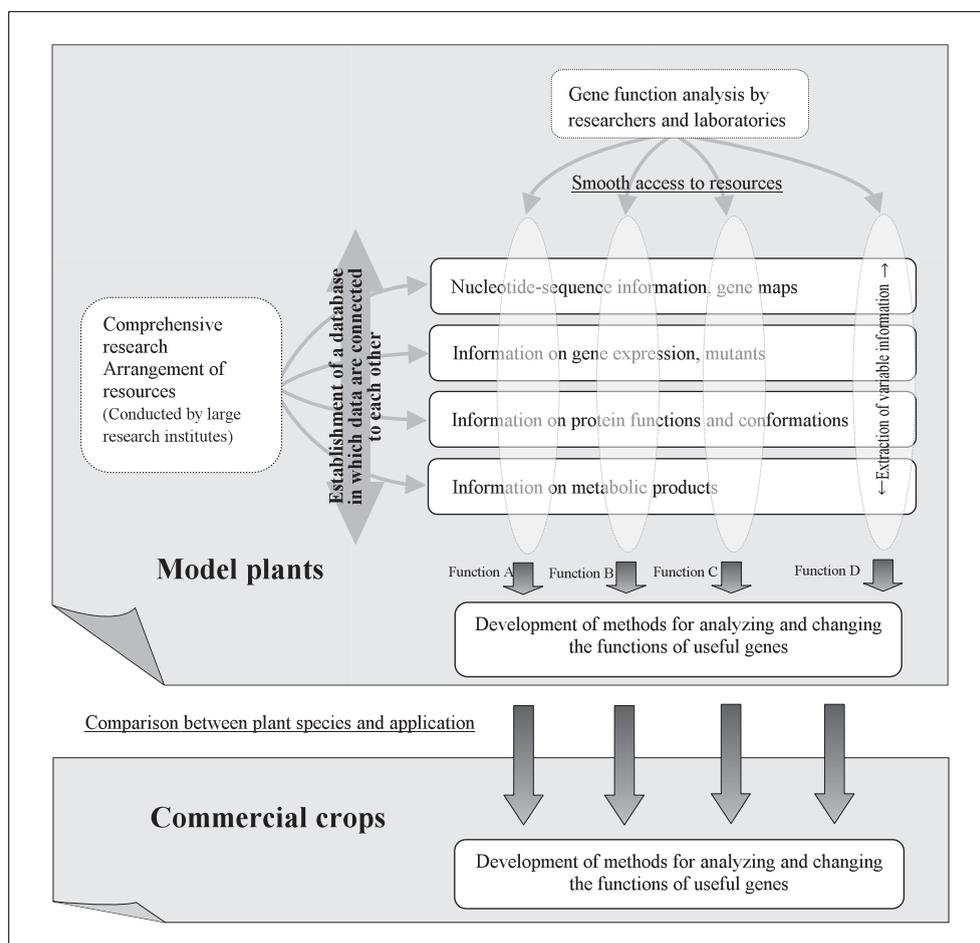
As seen in *Arabidopsis* studies, led by projects, plant molecular biology advances. First, large-scale and comprehensive sequencing projects are

launched on plants whose experimental methods are established. Then, detailed analysis of each gene is conducted.

In such large-scale and comprehensive sequencing projects, along with whole genome sequencing, bioresources necessary for gene function analysis are prepared thoroughly by, for example, generating various mutants with T-DNAs\*<sup>8</sup> and transposons.\*<sup>9</sup> In these projects, only a few research institutes or groups of considerable size usually conduct steps from genome sequencing to arrangement of bioresources. Results of gene function analyses of model plants of especially Poaceae and Fabaceae can be promptly used in applied research like the development of crop varieties. Thus, these few institutes have to be careful in disclosing research information and offering bioresources to others.

On the other hand, university researchers and laboratories have clarified the detailed functions of each gene using genome sequence information and various gene resources. As described in

Figure 7: Framework for the promotion of plant molecular biology



Source: Authors' own compilation

Chapter 3.3, plant gene analysis usually proceeds from screening of mutants with mutations in targeted genes. Therefore, data and bioresources should be managed so that researchers in Japan can have smooth access to whatever is necessary for their studies (Figure 7).

Meanwhile, data formats in comprehensive analysis are becoming diversified. They include not only nucleotide sequence information but also other formats like gene expression profiles\*<sup>11</sup> obtained by microarray.\*<sup>10</sup> Laboratories and researchers of universities need to extract variable information related to the function of the targeted gene from such assorted data and gene resources. Thus, a database that can be used conveniently and in which relevant data are connected to each other needs to be established, led by specialists on bioinformatics with consideration of the opinions of others such as university laboratories about data disclosure.

### 3.5.2 Difference between plant and human genome research in the post-genome era

Gene function analysis in plant molecular biology has been accomplished as characterized in Chapter 3.3, in which progress in plant morphogenesis was described. First, bioresources such as mutants are stocked thoroughly. Then, mutants that seem to have mutation in the targeted gene are screened on the basis of their phenotypes, targeted genes are identified from mutant genomes, and experiments such as function recovery by gene transfer are conducted to assess the gene function (Figure 8). Such procedure has been common in plant molecular biology until now.

Steps from gene manipulation such as gene

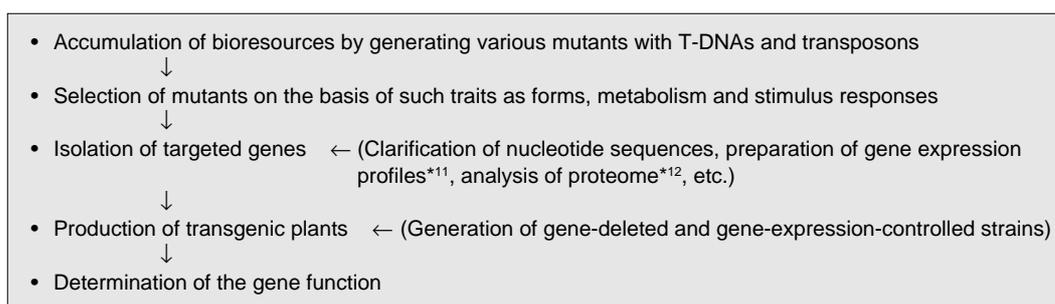
destruction to generation of mutants are much easier in plants than in animals because each plant cell is totipotent. Moreover, since a number of offspring can be obtained by crossing, it is effective even now to use such methods as map-based cloning in which candidate genes are found from a large-scale population by using DNA markers and natural recombination of chromosomes.

Meanwhile, in human post-genome researches, experiments in which human genes are directly manipulated are impossible. Moreover, even if, for example, a knockout mouse is necessary for gene function analysis, its production in a laboratory is impossible at the current general technological level. Unlike plants, comprehensive generation of animal mutants is quite difficult. Consequently, demands for research in which the function of protein is assessed from its conformation analysis and, thus, the biological mechanism is understood will increase more in animals than in plants.

Although whole genomes have been sequenced in several plants, only a few gene functions have been clearly revealed. Therefore, gene function analysis by using diverse mutants will be promoted further in plant research in the post-genome era. Plant and human genome researches in the post-genome era have different methodologies, so it is important to pursue research using the advantages of experimental methods peculiar to plants.

In the meantime, knowledge about plant morphology and physiology and the talent of such institutes as agricultural experiment stations, which have bred crops, will play a crucial role in steps like “Selection of mutants on the basis of such traits as forms, metabolism and stimulus responses,” “Production of transgenic plants” and

**Figure 8:** Main steps of plant molecular biological research



Source: Authors' own compilation

“Determination of the gene function” as shown in Figure 8.

### 3.5.3 Goals to be reached and the present research level in plant molecular biology

There is much hope that plant molecular biology will help solve the problems associated with an increasing population, food shortages caused by the decline and desertification of farmland, and environmental issues such as global warming and environmental pollution by various chemicals. As shown in Table 3, plants are being developed through the application of achievements in plant molecular biology.

According to a survey by the International Service for the Acquisition of Agri-biotech Applications (ISAAA), an international nonprofit organization, the total cultivated area of genetically modified plants in the world exceeded 50 million hectares in 2001 for the first time. It reached 52.6 million hectares, or 3.8% of the world’s cultivated acreage of 1.38 billion hectares, surging 19% from the previous year. However, only a few plants have become widespread so far, as seen in the fact that herbicide-resistant soybean, maize and cotton account for 77% of the GM-plant-cultivated area and Bt plants, or pest-resistant plants into which genes encoding insecticide proteins are introduced, account for 15%. In these herbicide-resistant and Bt plants, not genes in plant genomes but genes isolated from microbes were transferred to add new functions.

As in the research on drought-resistance acquisition described in Chapter 3.3, technology

for revealing plant gene functions and introducing a desired function into a plant has developed just recently along with the progress in *Arabidopsis* genome studies. We have just reached the stage in which we can evaluate the effectiveness of the technology by conducting field tests and adding various improvements. Similarly, we need to thoroughly analyze the numerous genes involved in a plant’s basic functions such as metabolism and signal transmission, and introduce desired traits into targeted plants by applying these functions. Such technique will realize the development of plants, such as high-yielding plants, salt-resistant plants, drought-resistant plants, heavy-metal-absorbing plants, and NO<sub>x</sub>/SO<sub>x</sub> absorbing or decomposing plants, which will help to solve global issues like food and environmental problems.

Therefore, in order to achieve such higher goals as solution of global issues like food and environmental problems through plant molecular biology, we need to promote function analysis of genes related to desired plant traits making good use of model plants like rice and *Arabidopsis*. In this way, we will broaden our understanding of higher plants at the molecular level.

## 3.6 Conclusion

In December 2000, an international consortium of Japan, the U.S. and the U.K. reported to Nature that it completed *Arabidopsis* genome sequence for the first time in higher plants. In April 2002, Syngenta and the Beijing Genomics Institute reported to Science that they separately

**Table 3:** Types of genetically modified plants with respect to their purposes

Plant types	Added properties
Plants benefiting producers	Herbicide-resistant plants, pest-resistant plants, virus-resistant plants, high-yielding plants, salt-resistant plants, drought-resistant plants, etc.
Plants benefiting consumers	High-quality plants (e.g., high-oleic-acid plants), good-tasting plants, inexpensive plants, etc.
Plants for health care and medical treatment in developing countries	High-vitamin-A plants, plants for preventing infections, plants producing diagnostic medicine, etc.
Plants for recovering the environment	Heavy-metal-absorbing plants, NO <sub>x</sub> / SO <sub>x</sub> absorbing or decomposing plants, etc.
Others	Plants generating clean energy

Source: the figure made by Prof. Hiroshi Kamada of the Institute of Biological Sciences, University of Tsukuba

completed rice genome sequence. As the research basis for gene function analysis becomes firm, it is expected that gene function analysis of plants including commercial crops will become far more efficient and international competition in function analysis of useful genes will become severer.

Meanwhile, it is required of plant molecular biology to develop plants supporting the life of humanity while enhancing the safety and credibility of genetically modified plants, so that this field of study may help to solve global issues such as food and environmental problems.

Therefore, we must take advantage of the peculiarity of plant experiments, that is, the simplicity in generating bioresources necessary for gene function analysis by gene transfer, and exploit every tool in Japan related to agricultural and plant research. We must make fruits of plant molecular biological studies, the common property of humanity, as soon as possible.

#### Acknowledgements

Together with our findings, we have compiled this report based on the lecture "Plant Molecular Biology—Now and Future" given Professor Kiyotaka Okada of the Graduate School of Science, Kyoto University, on April 23, 2002.

We earnestly thank Professor Okada for providing us with valuable advice and the related data for the writing of this report.

#### Glossary

\* 1 cDNA

A complementary DNA copy of mRNA, which carries the amino acid sequence of the protein.

\* 2 Tag line

Mutants produced by introducing DNA fragments with known sequences into the genome at random are called tag lines. Genes causing mutation can be isolated from

mutants with targeted phenotypes by analyses of the adjacent sequences using the introduced transgenes with known sequences.

\* 3 High-density gene linkage map

A map showing gene locations in the genome with high density by calculating the distances between genes from natural recombination values.

\* 4 Expressed sequence tag (EST)

A partial sequence of a cDNA.

\* 5 DNA marker

A distinctive nucleotide sequence whose location in the genome is identified.

\* 6 Map-based cloning

A method for identifying gene location in the genome by using DNA markers located near the targeted genes.

\* 7 Transcription factor

A factor controlling gene expression.

\* 8 T-DNA

Agrobacterium, a bacterium infecting plants, has a Ti plasmid that is a circular DNA carrying a region called T-DNA. When an Agrobacterium infects a plant, its T-DNA is transferred to the plant's chromosome. Thus, T-DNA is used for gene introduction into plants.

\* 9 Transposon

A transposable genetic element that can move from a certain location to another location in the genomic DNA.

\*10 Microarray

An array in which various cDNAs are applied with high density to a slide glass. It enables detection of numerous genes at a time.

\*11 Gene expression profile

Data showing the temporal and spatial expression pattern of each gene.

\*12 Proteome

A set of protein relations in cells and tissues.