USE OF cDNA MICROARRAY TECHNOLOGY FOR IDENTIFICATION OF NOVEL GENES RESPONDING TO ABSCISIC ACID PHYTOHORMONE

D. Cabezas, Sandra Pérez✉, R. Huelva, Dong Haitao y Li Debao

ABSTRACT. DNA microarray technology is a new and powerful technology that will substantially increase the speed of molecular biological research. This paper gives a survey of DNA microarray technology and its use in gene expression. Studies of 4370 unigenes from a library of rice endosperm and leaf tissue were used to detect the expression levels of mRNA from rice stem tissue treated by water and plant hormone ABA. Results showed that the expression levels of five genes were depressed by phytohormone ABA. Reverse Northern blotting confirmed that one of the five genes H024g06 was really depressed by ABA. Bioinformatic analysis showed that the gene H024g06 is the same as the cytochrome C gene. Previous researches revealed that the cytochrome C gene was related to such stress responses as drought and coldness, while ABA could induce plant stress response. Therefore, these results suggest that the cytochrome C gene may have a certain mediating function during the stress response induced by ABA phytohormone.

Key words: molecular genetics, ABA, cytochrome C

RESUMEN. Se utilizó el cDNA microarreglo con 4370 unigenes, provenientes de la biblioteca del endospermo del arroz y de los tejidos de las hojas, para detectar los niveles de expresión del mRNA de los tejidos del tallo del arroz tratados con agua y con la hormona ácido abscísico (ABA). Los resultados mostraron que los niveles de expresión de cinco genes fueron reprimidos por la fitohormona ABA. El Reverse Northern confirmó que uno de los cinco genes (H024g06) fue realmente reprimido por el ABA. Los análisis bioinformáticos mostraron que el gen H024g06 es igual que el gen citocromo C. Investigaciones anteriores revelaron que el gen citocromo C estuvo relacionado con respuestas de estrés como la sequía y la frialdad, mientras que el ABA pudo inducir la respuesta del estrés de la planta. Por todo esto, los resultados sugirieron que el gen citocromo C puede tener cierta función de mediación durante la respuesta al estrés inducida por la fitohormona ABA.

Palabras clave: genética molecular, ABA, citocromo C

INTRODUCTION

cDNA microarray is a very useful tool with which it is possible to find some differences between population and gene expression level, to analyze plant hormone inducible gene and function, tissue-specific genes, target of transcription factors and new markers for various physiological processes involved in abiotic stress, response hormone signal transduction and pathogen attack (1). Using an Expressed sequence of tag (EST) has also provided a major contribution with the discovery of expressed genes (2, 3). The capability of the technology to act as a powerful tool to link gene to function will no doubt aid future research into plant functional genomics.

The abscisic acid (ABA) phytohormone is probably present in all higher plant forms. In developing seeds, ABA is necessary for inducing the synthesis of reserve proteins and lipids as well as for the onset of seed dormancy and the acquisition of desiccation tolerance. Endogenous ABA levels peak during seed maturation and the onset of primary dormancy. ABA also plays important roles in vegetative development in response to various environmental stresses such as drought and high salinity conditions. Moreover, ABA is known to control the expression of many genes related to these phenomena (4). Thus, an understanding of the mechanisms underlying the regulation of plant ABA levels is a crucial part of determining ABA action in plant growth and physiological responses, which is correlated to endogenous ABA levels. Since the rates of synthesis and breakdown determine ABA levels *in situ*, both mechanisms should be studied in detail, although the rate of breakdown remains largely unknown (5). The recent glut of molecular, genetic and biochemical studies on ABA biosynthesis has allowed remarkable breakthroughs towards understanding the regulatory mechanisms associated with each gene or enzyme (6, 7, 8, 9).

Studying the phytohormone can contribute to our understanding of the molecular functioning of ABA in the guard cell and, more generally, to our concepts that the plant may respond differently to a given hormone in a concentration-dependent manner (10).

Ms.C. D. Cabezas y Ms.C. Sandra Pérez, Profesores del Departamento de Protección de Plantas, Ms.C. R. Huelva, Profesor del Departamento de Química, Universidad Agraria de La Habana, San José de las Lajas, La Habana, Cuba; Dr. Dong Haitao y Dr. Li Debao, Profesores Asistentes de Bioinformática y del Grupo de Investigaciones de Redes Génicas, Huajia Pool Campus, Universidad de Zheijiang, Hangzhou, 310029, China

✉ sandraperez_2000@yahoo.com

In this experiment a cDNA microarray including 4370 genes from rice (*Oryza sativa*) cDNA libraries was produced. It was employed to identify and clone novel genes responding to ABA hormone. Also, this technology was used to find tissue-specific genes expressed in rice endosperm, stem and leaf respectively.

MATERIALS AND METHODS

Plant materials and RNA isolation. The seeds of three rice (*Oryza sativa*) lines, Aijiaojian, Luzaofeng and ZhenchangA, were sown in basin, 50 mg.L^{-1} of the ABA phytohormone was applied to rice seedling with foliar spraying at 3-leaf stage. Water was applied as control. After germination, stem and leaf tissues of the rice line were sampled and stored at -70ºC at 4-leaf stage.

All the samples were harvested and ground to a fine powder in liquid nitrogen and then the samples were homogenized in 1 mL of Trizol reagent (GIBCO-BRL) per 50-100 mg of tissue. Intervals of 0, 9, 20 and 32 h were used for isolated total RNA, according to the manufacturer's protocol described at Life Technologies.

Preparation of template DNA for PCR. Plasmid derivatives of pSPORT^{TM} (GIBCO/BRL. Company) were used as templates in PCR reactions. The plasmids were either present in crude bacterial lysate or purified by the mini-preparation kit using multi-screen 96-well filter plate. The bacterial lysate was prepared from single colonies of plasmid-containing *Escherichia coli* XL1-Blue [Stratagene]. The lysate was stored at -20°C and used without further purification as template in PCR reactions. 10 µL of lysate were used as template in a 50-µl PCR reaction mixture. Oligonucleotides 5'GTAAAACGACGGCCAGTGATTT3' and 5'CAGGAAACAGCTATGACCATG3' were used in 40-cycle PCR reactions (94ºC, 5 minutes; 94ºC, 45ºC; 57ºC, 1 minute; 72ºC, 2 minutes; the last cycle was at 72ºC, 10 minutes). In order to obtain DNA sequence information of plasmids present in crude bacterial lysate, *E. coli* XL1-Blue was transformed with lysate and the plasmids were purified from obtained trans-formants with the mini-preparation kit system.

Isolation of Poly A + mRNA from total RNA. Isolation of Poly A^{+} mRNA from total RNA was done by Oligotex mRNA Spin-Column, according to the manufacturer's protocol.

Construction of cDNA library. A cDNA library was made from endosperm, stem and leaves at 4-leaf stage. Total RNA was isolated with Trizol reagent [Life Technology] following the manufacture's instructions. Isolation of Poly A^{+} mRNA from total RNA was done by Oligotex mRNA Spin-Column, according to the manufacture's protocol. Double-stranded cDNAs were synthesized with the Universal RiboClone® cDNA Synthesis System [Promega]. cDNA libraries were planted on 1.5 % agar solid LB medium (pH 7.0) with 100 µg.mL^{-1} Ampicillin and inoculated in 37ºC overnight. After the colonies appeared, a single clone was picked up and transferred to each well of a 96Square-Well block with 1mL of growth medium 2YT that was prepared at pH 7.0 (Table III), and contained the appropriate selective agent. The 96Square-Well block was incubated for 20-24 hours at 37ºC, and shaken at 200 rpm.

The fragments of approximately 0.3-1 kb were cloned into plasmid PBS-SK^{+} and used to transform *E. coli* XLI-Blue. Inserts were amplified by PCR and the DNA fragments obtained were precipitated with ethanol.

Preparation of total cDNA probes. 0.1 µg mRNA was Reverse Transcription labeled: 5 µL of mRNA, 5 µL of Oligo dT (100 µg.µL^{-1}) 18-mer and 2 µL of 6N Random Hexomeric Primer (100 µg.µL^{-1}). The sample was heated at 70°C for five minutes and placed on ice for five minutes too, and then 10 µL of 5x first strand buffer, 5 µL of 0.1M DTT, 3 µL of 10 mM dNTP (10mMdATP, 10mM dGTP, 10mM d TTP and 1mM dCTP), 5µL of a-32 P-dCTP, 2µL of M-MLV (200 unit/µL), and 4µL of DEPC-H2O were added.

Sequence analysis. Plasmid DNA was extracted by mini-preparation using a multi-screen 96-well filter plate and subjected to a sequence. The DNA sequence was determined using the dye terminator cycle sequencing method with a MegaBase 1000 (Amershan Pharmacia Biotech. Inc). The sequence data was kicked off by software chromas and redundant sequence was compared with software DNA tools.

Array preparation and chip hybridation. The denaturation product was transferred from 96-well plates to 384 will plates and the plates were placed in genome solution (Gene TAC ^{TM} G ^{3}, Germany) for array, and after completed the spotting, it was taken up by the membrane and put it into UV-Scrssliker (UVP. Inc. USA) a UV dose of 6000 microjoules.

Chip membranes were placed in tubes and washed with 3 mL of 0.25 M NaPO4 buffer, mixed well and discarded them, then a 5-6 mL of pre-hybridization solution was added. The tubes were placed for two hours at 60-65ºC in a Biometra OV2 and then the probes were mixed, added to the tube and placed for 10-12 hours at 60-65ºC in a Biometra OV2.

After hybridization, the solution was discarded inside the tube and a solution A (Table I) was added. The tubes were placed on Biometra OV2 for five minutes at 60ºC, a solution A was then discarded and the tubes were placed on Biometra OV2 for five minutes. A solution B (Table I) was added to the membrane for washing until the monitor shows from 3 to 5. If the monitor did not register between 3 and 5, the membranes need to be washed two more times with the same solution for a period of five minutes. Membranes were then dried at room temperature.

Table I. Washing solutions

Solutions	1L
A	2xSSC
	0.1xSDS
B	0.1xSSC
	0.1xSDS

Finally, membranes were placed in a cassette (auto-radio exposure) and X film placed on them for analyzing results. Membranes with an X film were placed at -20ºC for a period of five to seven days.

Reverse Northern. Total RNA was subjected to electrophoresis on a 1.2 % (wv) formaldehyde agarose gel and transferred to a high-bond-N nylon membrane

(Amersham) using 20SSC (11). Blots were hybridized with the radio-labeled gene-specific probe for 16 h at 65°C. The blots were then washed once in 0.1 % SDS, 2SSC for 20 minutes at 65°C and twice in 0.1 % SDS, 0.1SSC for 20 minutes at 65°C, and exposed to X-ray film. To ensure the equal loading of RNA in individual lanes, gels were stained with ethidium bromide for comparison of the intensities of rRNA bands.

Bioinformatic analysis. The differential display gene sequencing was done again from 3' end and 5' end by the counting of two sequences, to get a full-length sequence. The full-length sequence was used for bio-informatic analysis including BLAST analysis, electronic PCR location and so on.

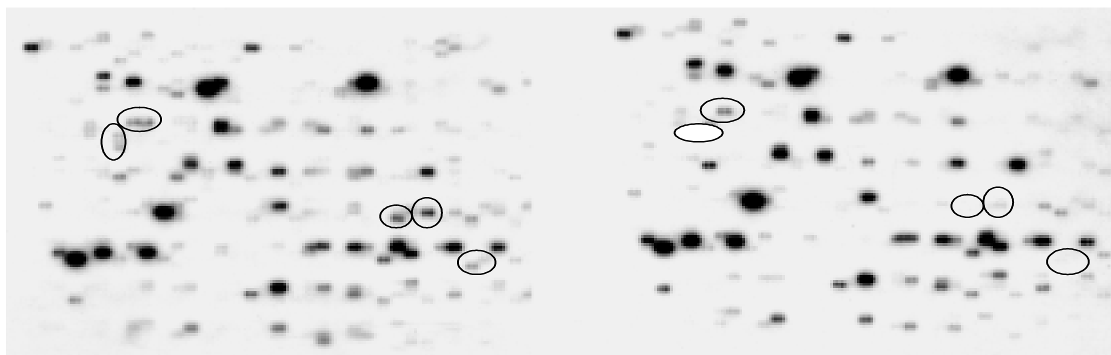
RESULTS AND DISCUSSION

Microarray were used for expression quantitatively level of 4370 unigenes from library of rice endosperm and leaf tissues were picked at random from cDNA libraries, amplified by polymerase chain reaction (PCR), and arrayed in duplicate chip membranes by using a robotic printer device. The rice endosperm cDNAs were arrayed together with the rice leaf tissues cDNAs, to assess the specificity of hybridization assay used to detect the expression level of mRNA from stem tissue of rice treated by water and plant hormone ABA.

Figure 1 shows the microarray after hybridization comparing two treatments and demonstrating all the positive hybridized cDNA clones; five cDNA clones were inhibited by phytohormone ABA at 20 hours in the rice line ZhenchangA. During the rest of the used time and rice line, no significative results were found.

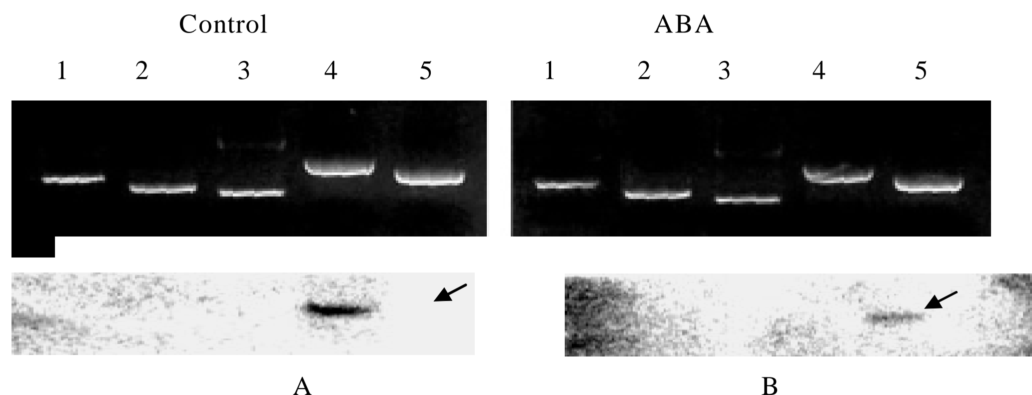
The five cDNA clones whose expression was inhibited by ABA, were amplified with PCR, and transferred to two nylon membranes. One was hybridized with probes made from mRNA prepared from water-treated rice stem tissue, the other was hybridized with one treated with ABA. The Reverse Northern blot results were shown in Figure 2.

From Figure 2, among the five genes whose expression was inhibited by ABA phytohormone in rice line ZhenchangA, H024g06 gene had a higher expression in water-treated stem tissue than that treated with ABA, which showed gene expression was exactly inhibited by ABA. This result confirmed the credibility of our cDNA chip hybridization results. The other four genes were not indictable in the hybridization. The possible reasons may lie in expression level of the four genes, which was too low to be detected, or it may be due to its inferior sensitiveness to the cDNA chip hybridization system.



CK: Rice control treated by water ABA: Rice induced by ABA plant hormone Differentially expressed cDNA sports were shown by circles

Figure 1. Microarray analysis of expression level of rice stem tissue induced by water (control) and plant hormone ABA (20 h)



Different positive candidates (lane 1-5) were amplified with M13 reverse and forward primers
Differential expressed band was shown with arrow
Panel A,B: Hybridized with cDNA probe prepared from rice induced by water (control) and plant hormone ABA

Figure 2. PCR amplification (up) and Reverse-Northern (bottom)

The five differentially displayed genes were retrieved and amplified. The electrophoresis of PCR products were performed before this product was transferred to the nylon membranes. The prepared nylon membranes were used for hybridization.

In Table II, it is possible to see the clone whose expression had been confirmed by the Reverse Northern blot; this clone was sequenced from 3'end and 5'end, and the full-sequence was obtained. The sequence was Blasted with all ESTs in rice EST database. The gene H024g06 sequence had a 99 % of similarity with that of cytochrome C gene in rice (Table III), and had a rather low similarity with the other sequences. So, a conclusion can be drawn that the candidate H024g06 gene is cytochrome C gene.

The inquiring result from Pubmed Blast for cytochrome C gene suggested that this gene is associated with several stress responses in plants (such as water/drought stress, and so on), and the expression level can also be inhibited by these stress responses (12). Research shows that ABA phytohormone could also induce light stress response (12). In this research, it was found for the first time that cytochrome C gene is involved in the response to ABA treatment, but the elucidation of the mechanism requires further work.

Cytochrome C is a small protein found loosely associated with the inner membrane of the mitochondrion. It is a soluble protein, unlike other cytochromes and it is an essential component of the electron transfer chain. It is capable of undergoing oxidation and reduction, and does not bind oxygen. It transfers electrons between Complexes III and IV. It is a highly conserved protein across the spectrum of species, found in plants, animals, and many unicellular organisms. This, along with its small size (molecular weight about 12,000 daltons), makes it useful in studies of evolutionary divergence (13).

The other four genes, whose expressions were inhibited by ABA in stem tissue treated with water or ABA, did not show any hybridization signals. One possible reason is their low expression level, and the technology of Reverse Northern blot is not as sensitive as cDNA microarray. All the results mentioned above showed that cDNA microarray is really a powerful tool in monitoring the fluctuation of expression levels of thousands of genes simultaneously. It could also act as a useful process in the overall study of plant development, gene regulation and expression, adverse environmental stress and mutation mechanisms, etc.

Table II. Counting of H024g06 sequences from 3'end and 5'end

```
•  3' h024g06 to 5' h024g06
•  CLUSTAL W (1.74) multiple sequence alignment        01-2-24 20:17:40
•  3' H024G06.TXT    ------------------------------------------------------------
•  5' H024G06 .TXT   AAGTATGACATGATTAGCCAAGCTCTAATACGACTCACTATAGGGAAAGCTGGTACGCCT
•  3' H024G06.TXT    ------------------------------------------------------------
•  5' H024G06 .TXT   GCAGGTACGGTCCGGAATTCCCGGGTCGACCCACGCGTCCGAAAAGAGAGTCGTAGTTCG
•  3' H024G06.TXT    ------------------------------------------------------------
•  5' H024G06 .TXT   CCTCTTCTTCCTCCTCTCGTTCTCGCGGCGGCGGCGGAGATGGCGTCGTTCTCGGAGGCT
•  3' H024G06.TXT    ------------------------------------------------------------
•  5' H024G06 .TXT   CCCCCGGGGCAACCCCAAGGCCGGCGAGAAGATCTTCAAGACCAAGTGCGCCCAGTGCCA
•  3' H024G06.TXT    ----------------------------------------------TGTCTGTTTGGAAG
•  5' H024G06 .TXT   CACCGTCGACAAGGGCGCCGGCCACAAGCAAGGTCCAAACTTGAATGGTCTGTTTGGAAG
•                                                                  **************
•  3' H024G06.TXT    -CAGTCAGGTACCACCC-TGGTTATTCCTACTCTACGGCCA-CAAGAACATGGCTGTGAT
•  5' H024G06 .TXT   GCAGTCAGGTACCACCCCTGGTTATTCCTACTCTACGGCCAACAAGAACATGGCTGTGAT
•                     **************** *********************** ******************
•  3' H024G06.TXT    CTGGGAGGAGA-CACACTTTATGACTACCGTGCTTAATCCTAAGAAGTACATCC-TGGAA
•  5' H024G06 .TXT   CTGGGAGGAGAACACACTTTATGACTACC-TGCTTAATCCTAAGAAGTACATCCCTGGAA
•                    *********** *****************  ************************ *****
•  3' H024G06.TXT    CCAAGATGGTCTTCCCCTGGTTGAAGAAGCCACAGGAGCGTGCTGATCTTATGTTCCTAC
•  5' H024G06 .TXT   CCAAGATGGTCTTCCCTGGGTTGAAGAAGCCACAGGAGCGTGCTGATCTTAT-TTCCTAC
•                    ****************  ********************************** *******
•  3' H024G06.TXT    CTGAAGGAAGCAACCTCTTAAAAGGAGTGGAGCTGTCGTAACATGAGACAATCGGACA-T
•  5' H024G06 .TXT   CTGAAGGAAGCAACCTCTTAAAAGGAGTGGAGCTGTCGTAACATGAGACAATCGGACAAT
•                    ********************************************************** *
•  3' H024G06.TXT    TCATTCTACAGTTAAATAAAAATATTTAGACATCTGGGTCGCTCTGGTTTTACAGGGTGC
•  5' H024G06 .TXT   TCATTCTACAGTTAAATAAAAATATTTAGACATCTGGGTCGCTCTGGTTTTACAGGGTGC
•                    ************************************************************
•  3' H024G06.TXT    AATCCACCGAGACA-TTATCTTGTTATATCCTTCGTTCTTATGGTTTATGGGTTCCATAT
•  5' H024G06 .TXT   AATCCAC-GAGACAATTATCTTGTTATAT---TTGTTCTTATGGTTTATGGGTTCCATAT
•                    ******* ****** **************   * **************************
•  3' H024G06.TXT    CTTAGGTTTAGCAGGAAAAGTCTTTCACAGCTTTTGCTTTAACTGCTGGAAAACACCAGT
•  5' H024G06 .TXT   CTTAGGTTTAGCAGGAAAAGTCTTTCACAGCTTT--GTTTAACTG-TGGAAAACAC-AGT
•                    **********************************   ******** ********** ***
•  3' H024G06.TXT    ATTTTGATCACCAGTCGGTGAGACTGGATTGCATCCCTTTTGGAAAAATACTGAGATCCC
•  5' H024G06-.TXT   ATTTAGATCAC-AGTNGGTGAGACTGGAT-GCATCC--TTTGGAAAA-TA-TGAGGATCC
•                    **** ****** *** ************* ******  ********* ** ****   **
•  3' H024G06.TXT    TTAAATTTGTGATATTTTGATCCAAAAAAAAAAAAAAA--------------------
```

Table III. Relativity analysis between H024g06 clone and cytochrome C gene

```
BLASTN 2.1.2 [Nov-13-2000]
Query= h024g06     (816 letters)
Database: riceseq          175,006 sequences; 163,192,531 total letters
dbj|D12634.1|RICYCYTC  Rice mRNA for cytochrome C, complete cds  1197  0.0
 Length = 654

Query: 155 cggagatggcgtcgttctcggaggctcccccggggcaaccccaaggccggcgagaagatc 214
           |||||||||||||||||||||||||||| || ||||||||||||||||||||||||||||
Sbjct: 1   cggagatggcgtcgttctcggaggctcc-cccgggcaaccccaaggccggcgagaagatc 59

Query: 215 ttcaagaccaagtgcgcccagtgccacaccgtcgacaagggcgccggccacaagcaaggt 274
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 60  ttcaagaccaagtgcgcccagtgccacaccgtcgacaagggcgccggccacaagcaaggt 119

Query: 275 ccaaacttgaatggtctgtttggaaggcagtcaggtaccacccctggttattcctactct 334
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 120 ccaaacttgaatggtctgtttggaaggcagtcaggtaccacccctggttattcctactct 179

Query: 335 acggccaacaagaacatggctgtgatctgggaggagaacacactttatgactacctgctt 394
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 180 acggccaacaagaacatggctgtgatctgggaggagaacacactttatgactacctgctt 239


Query: 395 aatcctaagaagtacatccctggaaccaagatggtcttccctgggttgaagaagccacag 454
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 240 aatcctaagaagtacatccctggaaccaagatggtcttccctgggttgaagaagccacag 299

Query: 455 gagcgtgctgatcttatttcctacctgaaggaagcaacctcttaaaaggagtggagctgt 514
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 300 gagcgtgctgatcttatttcctacctgaaggaagcaacctcttaaaaggagtggagctgt 359
Query: 515 cgtaacatgagacaatcggacaattcattctacagttaaataaaaatatttagacatctg 574

           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 360 cgtaacatgagacaatcggacaattcattctacagttaaataaaaatatttagacatctg 419

Query: 575 ggtcgctctggttttacagggtgcaatccaccgagacaattatcttgttatatccttcgt 634
           ||||||||||||||||||||||||||||| ||||||||||||||||||||||  || ||
Sbjct: 420 ggtcgctctggttttacagggtgcaatcca-cgagacaattatcttgttatat--tttgt 476

Query: 635 tcttatggtttatgggttccatatcttaggtttagcaggaaaagtctttcacagcttttg 694
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 477 tcttatggtttatgggttccatatcttaggtttagcaggaaaagtctttcacagcttttg 536

Query: 695 ctttaactgctggaaaacaccagtattttgatcaccagtcggtgagactggattgcatcc 754
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 537 ctttaactgctggaaaacaccagtattttgatcaccagtcggtgagactggattgcatcc 596

Query: 755 cttttggaaaaatactgagatcccttaaatttgtgatattttgatcc 801
           ||||||||||||||||||||| |||||||||||||||||||||||||
Sbjct: 597 cttttggaaaaatactgagat-ccttaaatttgtgatattttgatcc 642
```

Query: Sequence of H024g06 clone; *Subject*: Rice sequence form Database

In this study, the expression of cytochrome C gene is found to be inhibited by ABA phytohormone. Previous research showed that the expression of this gene was also inhibited by many adverse environmental stresses (12), such as drought and cold. ABA phytohormone treatment is also associated with adverse environmental stress responses. Cytochrome C has some relationship with another process in the plant, since this is the terminal enzyme of the mitochondria respiratory chain catalyzing electron transfer from cytochrome C to molecular oxygen (14). The molecular mechanism of this process is not yet understood. At present, little is known about such important structural features, as the position of the prosthetic groups or the location and characteristics of the cytochrome C binding sites in the cytochrome C oxidase complex (15).

The rate of oxidation of cytochrome C catalyzed by cytochrome C oxidase, which is the final enzyme of the mitochondrial respiratory chain (16), is also the terminal electron transfer component of the mitochondrial electron-transfer chain and a site of energy coupling.

Cytochrome C is a well-characterized protein component of the cytochrome c-f complex, which plays an important role in the electron transfer of photosynthesis reaction (17).

The results of this study suggest that further research should be carried out to determine whether the cytochrome C gene is involved in adverse environment stress response induced by ABA.

The results of this experiment demonstrate that our cDNA microarray system can be applied practically to monitoring the transcript abundance of tissue-specific expressing genes. Using this system, we successfully cloned the genes whose transcript activity was inhibited by ABA phytohormone. The hybridization results were confirmed by using Reverse Northern blot.

As the development of molecular biology, increasing attention is being paid to uncovering DNA or cDNA sequence expression variations among groups of individuals as well as between different tissues, so microarrays come to be one of the most important tools for genomic research. It is used on many aspects: cDNA microarrays are capable of profiling gene expression patterns of tens of thousands of genes in a single experiment; so, it could be used to study gene expression on a large scale. They can also be used to monitor changes in gene expression in response to various environmental conditions including hormone and other treatments. Microarray potentially allows rapid and cost-effective screens for all possible mutations and sequence variations in genomic DNA and so on (18).

The effect of ABA phytohormone on the transcripting activity of genes in rice was determined using our cDNA microarray system. An association between cytochrome C gene and stress response was found through these experiments. This suggests that cytochrome C gene can be involved in the stress response induced by ABA.

REFERENCES

1. Seki, M.; Narusaka, M.; Kasuga, M.; Yamaguchi-Shinozaki; Carninci, P.; Hayashizaki, Y. and Shinozaki, K. Monitoring the expression pattern of 1300 Arabidopsis genes under drought and cold stress by using a full-length cDNA microarray. *Plant Cell*, 2001, vol. 13, p. 61-72.
2. Cooke, R.; Laudie, M.; Grellet, F.; Delseny, M. and Morris, P. C. Further progress towards a catalogue of all Arabidopsis gene analysis of a set of 5,000 non-redundant ESTs. *Plant Journal*, 1969, vol. 9, p. 101-124.
3. Asamizu, E.; Nakamura, Y.; Sato, S. and Tabata, S. A large scale analysis of cDNA in *Arabidopsis thaliana*, generation of 12,028 non-redundant expressed sequence tags from normalized and size-selected cDNA library. *DNA Research*, 2000, vol. 7, p. 175-180.
4. Seo, M. and Koshiba, T. Complex regulation of ABA biosynthesis in plants. *Trends Plant Science*, 2002, vol. 7, no. 1, p. 41-48.
5. Cutler, A. J. and Krochko, J. E. Formation and breakdown of ABA. *Trends Plant Science,* 1999, vol. 4, p. 472-478.
6. Zeevaart, J. A. D. Abscisic acid metabolism and its regulation. In Biochemistry and Molecular Biology of Plant Hormones. New York : Elsevier, 1999, p.189-207.
7. Liotenberg, S. /*et al.*/. Molecular biology and regulation of abscisic acid biosynthesis in plants. *Plant Physiology Biochemistry*, 1999, vol. 37, p. 341-350.
8. Taylor, I. B.; Burbidge, A. and Thompson, A. J. Control of abscisic acid synthesis. *Journal of Experimental Botany*, 2000, vol. 51, p. 1563-1574.
9. Milborrow, B. V. The pathway of biosynthesis of abscisic acid in vascular plants: a review of the present state of knowledge of ABA biosynthesis. *Journal of Experimental Botany*, 2001, vol. 52, p. 1145-1164.
10. Alain, C. and Alain, V. Two potential Ca^{2+}-dependent transduction pathways in stomata closing in response to abscisic acid. *Plant. Physiology and Biochemistry*, 1998, vol. 36, p. 257-262.
11. Sambrook, E. F.; Fritsch, T. and Maniatis, T. Molecular cloning: A Laboratory Manual. 2 Ed. Cold Spring Harbor Laboratory Press, 1989. 750 p.
12. Dhage, A. R.; Desai, B.; Naik, R. M.; Munjal, S. V. and Naik, M. S. Modification of the redox state of cytochrome C oxidase of rice due to certain stress treatments. *Indian Journal of Biochemistry and Biophysics*, 1992, vol. 29, p. 425-427.
13. Wikipedia Encyclopedia. Available in: <http://en.wikipedia.org/wiki/Cytochrome_C>.
14. Wikström, M.; Krad, K. and Saraste, M. Cytochrome oxidase-A Synthesis. *Annual Review of Biochemistry*, 1981, vol. 50, p. 623-658.
15. Kadenbach, B. and Merle, P. On the function of multiple subunits of cytochrome C oxidase from higher eukaryotes. *FEBS Letters*, 1981, vol. 135, p. 1-11.
16. Bruno, M. and Hans, R. Oxidation of Cytochrome C by Cytochrome C oxidase: Spectroscopic binding studies and steady-state kinetics support a conformational transition mechanism. *Biochemistry*, 1989, vol. 28, p. 244-252.
17. Nai-hu, W.; Jean, C. C. and Ray, W. Nucleotide sequence of rice cytochrome f gene and the presence of sequence variation near this gene. *Gene*, 1986, vol. 50, p. 271-278.
18. Aharoni, A and Vorst, O. DNA microarrays for functional plant genomic. *Plant Molecular Biology*, 2001, vol. 48, p. 99-118.

Recibido: 23 de mayo de 2003
Aceptado: 5 de agosto de 2004