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STUDIES ON THE GENE EXPRESSION IN DIFFERENT ALUMINUM-RESISTANCE PINEAPPLES

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ABSTRACT

Pineapple (*Ananas comosus* (L) Merrill) is one of an economically cultured crops in Taiwan. It is normally cultivated in soils containing strong acidity where aluminum (Al) often inhibits the growth of crop roots. This research was carried out with the objective of identifying the main genes that directly influence aluminum tolerance by resistant and non-resistant pineapples (Cayenne and Tainung No. 17, respectively). For this reason, both cultivars were grown in hydroponic nutrient solution, each containing 0 and 300 μM $AlCl_3$ in a growth chamber for four weeks. Samples of root apices were taken, followed by extraction of RNA, selection of primers, sequencing and processing of PCR. Finally, after examination of genes, the relative expressions of them for both cultivars were determined by quantitative PCR. The results obtained from polymerase chain reaction experiments proved the Al-resistance characteristics of pineapples are mainly regulated by two genes, namely GAPDH (glyceraldehyde-3-phosphate-dehydrogenase) and FBA (fructose biphosphate adenosine). This result may provide important indications for the study of advancing genetic mechanisms on Al-resistance in pineapples.
Additional keywords: *Ananas comosus*, real time-PCR, resistance genes, root apex

RESUMEN

Expresión genética en plantas de piña con diferente grado de resistencia al aluminio

La piña (*Anonas comosus* (L) Merrill) es uno de los cultivos económicamente importantes en Taiwán. Normalmente se cultiva en suelos que contienen una acidez fuerte donde el aluminio (Al) a menudo inhibe el crecimiento de las raíces. Esta investigación se realizó con el objetivo de identificar los principales genes que influyen directamente en la tolerancia al aluminio por piñas resistentes y no resistentes (Cayenne y Tainung No. 17, respectivamente). Para ello, las plantas se cultivaron en una solución hidropónica nutritiva que contenía $AlCl_3$ en concentraciones de 0 y 300 μM en una cámara de crecimiento durante cuatro semanas. Se tomaron muestras de ápices de raíces, lo cual fue seguido por un protocolo metodológico que incluyó extracción de ARN, selección de cebadores, secuenciación y procesamiento de PCR. Finalmente, después del examen de los genes, las expresiones relativas de los mismos para ambos cultivares se determinaron por PCR cuantitativo. Los resultados obtenidos mediante las pruebas de la reacción en cadena de la polimerasa mostraron que las características de resistencia al aluminio de las plantas de piña están regulados principalmente por los genes GAPDH (gliceraldehído-3-fosfato-deshidrogenasa) y FBA (fructosa bifosfato adenosina). Estos resultados pueden proporcionar indicaciones importantes para el estudio del avance de los mecanismos genéticos sobre la resistencia al aluminio en el cultivo de la piña.
Palabras clave adicionales: *Ananas comosus*, ápice radical, genes de resistencia, PCR en tiempo real

INTRODUCTION

Aluminum toxicity is already verified as one of environmental injuries that influence the growth of plant in the soils containing strong acidity. Le Van and Masuda (2004) used ten pineapple cultivars to proceed with an Al-resistance experiment, and found that Cayenne is the most Al-resistant cultivars. Chen and Lin (2010) evaluated different Al concentrations on four pineapple cultivars and found that Cayenne was the least affected, while Tainung No.17 was notoriously affected under high Al concentration. On the other hand, the nutrient absorption was increased and the cells of root apex were healthy in Cayenne after treating with 300 μM $AlCl_3$. However, nutrient absorption was inhibited and cells of root apices were injured in Tainung No.17 (Lin, 2010; Lin and Chen, 2019). So, the

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authors concluded that Cayenne is the most Al-resistant and Tainung No.17 is one of the most Al-sensitive cultivar.

Studies on Al-resistance genes of Arabidopsis, wheat and rice showed that the characteristics of resistant cells are related to their specific resistance genes (Degenhardt et al., 1998; Yin et al., 2009). Many proteins were identified for different crops (Salekdeh et al., 2002; Zhen et al., 2007), and pineapple (Chen and Lin, 2010) grown in unfavorable environments. However, few researches have been directed to Al-resistance genes of pineapples perhaps due to the fact that degree of resistance of plants in an unfavorable environment is a complicated phenomenon. It is closely related to genetics and paths of signals (Hoekenga et al., 2003).

Many current studies are directed toward molecular basics of resistance genes in order to improve growth of crops in strong acid soil. Although Al-resistance genes of many crops have been identified, such as wheat, soybean and Arabidopsis (Richards et al., 1994; Ragland and Soliman, 1997; Farh et al., 2017), the study of Al-resistance genes of some crops may not be applicable to other crops. That is the reason why genetics is worthy of further in-depth studies. Polymerase chain reaction (PCR) technique is a most commonly used, convenient and effective tool for gene identification (Mullis, 1990). This technique allows rapid and multiple growth of some section of special series of DNA (Marchuk et al., 1991). Following the identification of all genetic series of rice, the application of real-time PCR has been widely adopted for the study of gene transcription in many crops (Heid, 1996), including expressed analysis of genes related to environmental stress and metabolism of carbohydrates (Ishimaru et al., 2005; Kanegae et al., 2005).

For studying genes that were induced by Al toxicity, this technique was employed successfully to identify accurately many Al-resistance genes of rice, wheat and corn. These genes were found to be related to the composition of cell wall, metabolism of protein, secondary metabolism, anti-oxidizing environment and other Al-resistance function of the cell (Mao et al., 2004; Guo et al., 2007). The researches on genetics of Al-resistance pineapples are still rare so far. Chen and Lin (2010) studied the effect of applications high concentrations of aluminum in Cayenne pineapple and found a notorious variation on the protein metabolism. As Cayenne is Al-resistant while Tainung No.17 is not (Lin, 2010), the objective of this research was to use PCR technique to study the main genes as influenced factors on Cayenne and Tainung No. 17 pineapples under treatments including application or not of $AlCl_3$. The results may serve as a reference to future genetic studies on Al-resistance characteristics of pineapples.

MATERIALS AND METHODS

The preparation of pineapple samples. Fresh weight of about 81±8 g each of pineapple cultivars (Cayenne and Tainung No.17) were planted in planting pots (30 cm height, 25 cm inner diameter) that contained modified ingredients of Hoagland and Arnon (1950) hydroponic solution, and cultivated in a growth chamber. Plants in the containers were aerated by bubble driver. The environmental conditions were set at 28 ºC temperature/ 60 % relative humidity (13 hours at day-time), and 22 ºC temperature/ 80 % relative humidity (11 hours at night-time). Hydroponic solution was renewed every week. Each of the two pineapple cultivars was cultivated and treated with 0 and 300 μM $AlCl_3$ for four weeks.

Extraction of RNA and reverse transcription. Root apices of pineapple were extracted for RNA using Trizol total RNA isolation reagent (Ambion, Life Technologies) as protocol. The concentration of RNA was calculated by A-drop spectrophotometer. cDNA was synthesized from RNA using primers according to the Kit of cDNA reverse transcription protocol. The reactive system of reverse transcriptase contained RNA samples (100 ng), RT primer (50 nM), RT buffer (10X), dNTPs (100 mM), multiscribe reverse transcriptase (50 U/μl) and RNase inhibitor (40 U/μl) (RNaseOUT, Invitrogen). Then 20μl mixture was incubated in an Applied Biosystems 2700 thermal-cycler in a PCR reaction tube, and treated 10 min at 25 °C, 120 min at 37 °C, and 5 seconds at 85 °C. At last, it was maintained at 4 °C.

Selection of primers. The primers were chosen from GAPDH gene that were obtained from NCBI GenBank (GI:120669) sequences. The primer target sequences were advancely confirmed by Primer Express V.2.0 software. Primers GAPDH-

418F (5'- ACHGAYTACATGACHTAYATGTT-3') and GAPDH-907R (5'-CTTCCACCYCTCCA GTCYTT-3') showed the most suitable fragment of the GAPDH gene (489 bp).

Sequence of GADPH and FBA by proceeding of real-time PCR. The PCR reactions (25 μL) was proceeded using the method of Hasan et al. (2009), and the amplification was performed in an Applied Biosystems 2700 thermal-cycler. The gel was stained and observed with "Seeing Safe" and fluorescence illuminator (Dark reader, Clare Chemical Research). At last, the searching engine, basic local alignment search tool (BLAST) was used for the finding of accurate gene in the genebank. The primers of PCR products were cloned following the method of Hwa et al. (2011). Real-time PCR was proceeded by the method of Ma et al. (2012) which was first proposed by Mullis (1990). After PCR, dissociative curve was examined. The ΔΔCt method was determined for the quantitative expression of gene and the primer was A1+A-300GAPDH. The primer sequence for 95F was TTTGGCGAGAAGGCAGTCA, and for 300R was TCATTCACACCAACAACAAA CATC. The amino acid series of these proteins were used to determine the mRNA series, and then these mRNA series were reversibly transcribed to cDNA (complementary DNA). The cDNA was used commonly as primer and underwent a series of DNA polymerase chain reaction (PCR). The target genes were purified by genetic transplant, and the "specific genes" were subject to real-time quantitative PCR with primer design. Finally the genes were examined with PCR and their relative expressions in samples from root apices of Cayenne and Tainung No.17 after treating with non-Al and 300 μM $AlCl_3$ solution were determined by QPCR (quantitative PCR). Results are presented as images of the electrophoretic runs of PCR, and comparison of the relative expression of genes.

RESULTS AND DISSCUSION

Genetic Studies of Al-Resistant Pineapples. After examination with real-time PCR, it was confirmed the existence of genes GAPDH and FBA in Cayenne pineapple cultivar when exposed to 300 μM $AlCl_3$ solution (Figures 1 and 2). Apparent expression of GAPDH was observed in either Cayenne or Tainung No.17 pineapple cultivars treated or not with $AlCl_3$ solutions (Figure 1). The quantity of GAPDH expression was low for any cultivar without aluminum treatment, and therefore it was lower than that of the cultivars treated with the product. The ratio in GADPH of Tainung No.17 for 300 μM $AlCl_3$ is more than 20 times higher than that of non aluminum treatment. The ratio in the same gene for Cayenne is about 35 times higher than that without aluminum. These ratios are all much higher than 2 times which is usually considered high in relative expression. Thus the GADPH expression level for Cayenne is more prominent relative to Tainung No.17 (Table 1).

Similarly, apparent FBA expression was only found in Cayenne treated with 300 μM $AlCl_3$ (Figure 2). The ratio in the FBA relative expression level of Cayenne is about 20 times higher for Al treated plants relative to non treated plants. However, the ratio in FBA relative expression level of Tainung No.17 for 300 μM $AlCl_3$ was only about twice higher than that without aluminum, which means that FBA protein in the cultivar was inhibited under high Al concentration, although still showed higher expression than that with non Al treatment. It is concluded that the relative gene expression level of Cayenne is higher than that of Tainung No.17 and the quantity of FBA for Cayenne was about 35 times higher under aluminum treatment (Table 2). Iskandar et al. (2004) suggested that when the relative expressive level of a gene was higher than thirty, the expression of gene was apparent.

GAPDH has been widely distributed in the original nucleus and true nucleus of biota. It was thought to be related to glycolytic pathway, and when plants are in unfavorable environment, the GAPDH gene would regulate or modify in order to adapt to the environment (Hajitrzaei et al., 2006).

In a previous research, Chen and Lin (2010) studied proteins in root apices of Al-resistant Cayenne and found that GAPDH and FBA were the most apparent upregulated proteins when the plant was treated with high Al concentration. Similar results were observed by Chiadmi et al. (1999) on pea, and Mugford et al. (2010) on Arabidopsis.

In this study, GAPDH of Cayenne in Al-affected environment belonged to up regulated protein which had relatively higher quantity of

expression. This suggested that this gene might play an important role in metabolism under unforeseeable environments. Ohdan et al. (2005) and Narayanan et al. (2007) obtained comparative results when conducting genetic studies on Al-resistant rice.

It has been found that many altered proteins in the high Al concentration roots of Cayenne are associated closely with organic acids (Chen and Lin, 2010) which can help to maintain cell morphology and chelating of aluminum, and according to Le Van and Masuada (2004), the secretion of organic acids may reduce Al toxicity on root apices.

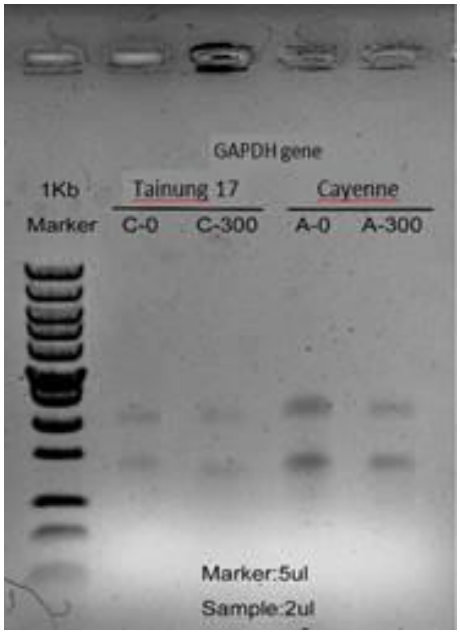

Figure 1. Gel electrophoresis of the RT-PCR products showing the expression of GAPDH genes in Cayenne and Tainung No.17 after treating with 0 and 300 µM $AlCl_3$

Table 2 indicates that the FBA gene experiment in Cayenne was extremely high relative to no Al treatment (Figure 2). This was attributed to internal modifications in the plant, and suggest that such response could protect cells of root apices of Al-resistant Cayenne in high aluminum environments.

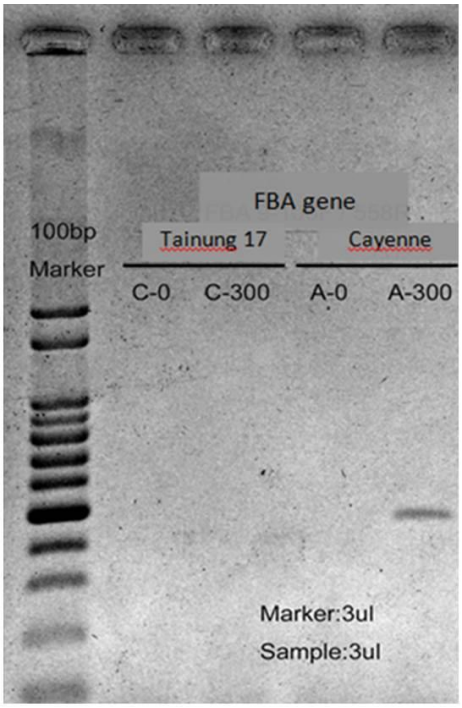

Figure 2. Gel electrophoresis of the RT-PCR products showing the expression of FBA genes in Cayenne and Tainung No.17 after treating with 0 and 300 µM $AlCl_3$

Table 1. The relative GAPDH gene expression by RT-quantity-PCR between Cayenne and Tainung No.17 pineapple with the treatments of 0 and 300 µM $AlCl_3$

Treatments	Ct (Target gene)	Ct (Reference gene)	ΔCt (Target)	ΔΔCt (Sample-Reference)	Relative expression level
Tainung No.17 (0 µM $AlCl_3$)	32.46	29.63	2.83	—	1.00
Tainung No.17(300 µM $AlCl_3$)	32.03	24.77	7.26	4.43	21.62
Cayenne(0 µM $AlCl_3$)	32.09	28.88	3.21	0.38	1.30
Cayenne(300 µM $AlCl_3$)	26.95	18.61	8.34	5.51	45.74

*GAPDH: glyceraldehyde-3-phosphate-dehydrogenase, Ct: threshold cycle number

Table 2. The relative FBA gene expression by RT-quantity-PCR between Cayenne and Tainung No.17 pineapple with the treatments of 0 and 300 μM $AlCl_3$

Treatments	Ct (Target gene)	Ct (Reference gene)	ΔCt (Target)	ΔΔCt (simple-Reference)	Relative expression level
Tainung No.17 (0 μM $AlCl_3$)	37.10	35.45	1.65	—	1.000
Tainung No.17(300 μM $AlCl_3$)	32.20	29.56	2.64	0.99	1.987
Cayenne(0 μM $AlCl_3$)	34.40	31.11	3.29	1.64	3.110
Cayenne(300 μM $AlCl_3$)	31.10	23.49	7.61	5.96	62.220

*FBA: fructose biphosphate adenosine, Ct: threshold cycle number

CONCLUSIONS

Aluminum resistance characteristics of studied pineapples cultivars are regulated at least by two genes: glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) and fructose biphosphate adenosine (FBA). Apparently, these genes could promote Al-resistance in Cayenne cultivar and protect cells of root apices of the plant under high aluminum environments. These results may serve as an important clue for genetics or mechanism of Al-resistance in pineapples.

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LITERATURE CITED

1. Chen, J.H. and Y.H. Lin. 2010. Effect of aluminum on variations in the proteins in pineapple roots. Soil Science and Plant Nutrition 56(3): 438-444.
2. Chiadmi, M., A. Navaza, M. Miginiac-Maslow, J.P. Jacquot and J. Cherfils. 1999. Redox signalling in the chloroplast: structure of oxidized pea fructose-1,6-bisphosphate phosphatase. The EMBO Journal 18(23): 6809-6815.
3. Degenhardt, J., P.B. Larsen, S.H. Howell and L.V. Kochian. 1998. Aluminum resistance in the Arabidopsis mutant alr-104 is caused by an aluminum-induced increase in rhizosphere pH. Plant Physiology 117(1): 19–27.
4. Farh, M.E.A., Y.J. Kim, J. Sukweenadhi, P. Singh and D.C. Yang. 2017. Aluminium resistant, plant growth promoting bacteria induce overexpression of aluminium stress related genes in *Arabidopsis thaliana* and increase the ginseng tolerance against aluminium stress. Microbiological Research 200: 45-52.
5. Guo, P.G., G.H. Bai and T.C. Brett. 2007. Transcriptional analysis between two wheat near-isogenic lines contrasting in aluminum tolerance under aluminum stress. Molecular Genetics and Genomics 277(1): 1-12.
6. Hajitrzaei, M.R., S. Biemelt and M. Peisker. 2006. The influence of cytosolic phosphorylating glyceraldehyde-3-phosphate dehydrogenase (GAPDH) on potato tuber metabolism. J. Exp. Bot. 57(10): 2363-2377.
7. Hasan, A.U., S. Suguri, J. Sattabongkot, C. Fujimoto, M. Amakawa, M. Harada and H. Ohmae. 2009. Implementation of a novel PCR based method for detecting malaria parasites from naturally infected mosquitoes in Papua New Guinea. Malaria Journal 8(1): 182-193.
8. Heid, C.A., J. Stevens and J.K. Livak. 1996. Real time quantitative PCR. Genome Research 6(10): 986-994.
9. Hoagland, D.R. and D.I. Arnon. 1950. The water culture method for growing plants without soil. California Agricultural Experiment Station. Berkeley, CA, USA. vol. 347. 32 p.
10. Hoekenga, O.A., T.J. Vision, J.E. Shaff, A.J.

Monfone and G.P. Lee. 2003. Identification and characterization of aluminum tolerance loci in Arabidopsis (*Landberg erecta* × *Columbia*) by quantitative trait locus mapping. A physiologically simple but genetically complex trait. Plant Physiology 132(3): 936-948.

11. Hwa, H.L., Y.Y. Chang, J.C. Lee, H.Y. Yin, L.H. Tseng, Y.N. Su and T.M. Ko 2011. Fourteen non-CODIS autosomal short tandem repeat loci multiplex data from Taiwanese. International Journal of Legal Medicine 125(2): 219-226.
12. Ishimaru, T., T. Hirose and T. Matsuda. 2005. Expression patterns of genes encoding carbohydrate-metabolizing enzymes and their relationship to grain filling in rice (*Oryza sativa* L.): comparison of caryopses located at different positions in a panicle. Plant Cell Physiology 46(4): 620-628.
13. Iskandar H.M., S. Robert, R.E. Simpson, R.E. Casu, G.D. Bonnett, D.J. Maclean and J.M. Manners. 2004. Comparison of reference genes for quantitative real-time polymerase chain reaction analysis of gene expression in sugarcane. Plant Molecular Biology Reporter. 22(3): 325–337.
14. Kanegae, H., K. Miyoshi, T. Hirose. 2005. Expressions of rice sucrose non-fermenting-1 related protein kinase 1 genes are differently regulated during the caryopsis development. Plant Physiology and Biochemistry 43(7): 669-679.
15. Le Van, H. and T. Masuda. 2004. Physiology and biological studies on aluminum tolerance in pineapple. Australian Journal of Soil Research 42(6): 699-707.
16. Lin, Y.H. 2010. Effects of aluminum on root growth and absorption of nutrients by two pineapple cultivars [*Ananas comosus* (L.) Merr.]. African Journal of Biotechnology 9(26): 4034-4041.
17. Lin, Y.H. and J.H. Chen. 2019. Effects of aluminum on the cell morphology in the root apices of two pineapples with different Al resistance characteristics. Soil Sci. Plant Nutr. 65(4): 353–357.
18. Ma, D.H., J.H. Lai, S.T. Yu, J.Y. Liu, U. Yang, H.C. Chen et al. 2012. Up-regulation of heat shock protein 70-1 (Hsp70-1) in human limbo-corneal epithelial cells cultivated on amniotic membrane: A proteomic study. Real-time PCR. Journal of Cell Physiology 227(5): 2030-2039.
19. Mao, C.Z., K.K. Yi and L. Yang. 2004. Identification of aluminium regulated genes by cDNA-AFLP in rice (*Oryza sativa* L.): Aluminium-regulated genes for the metabolism of cell wall components. Journal of Experimental Botany 55(394): 137-143.
20. Marchuk, D., M. Drumm, A. Saulino and F.S. Collins. 1991. Construction of T-vectors, a rapid and general system for direct cloning of unmodified PCR products. Nucleic Acids Research 19(5): 1154.
21. Mugford, S.G., C.A. Matthewman, L. Hill and S. Kopriva. 2010. Adenosine-5'-phosphosulfate kinase is essential for Arabidopsis viability. FEBS. Letters 584(1): 119-123.
22. Mullis, K.B. 1990. The unusual origins of the polymerase chain reaction. Scientific American 262(4): 56-65.
23. Narayanan, N.N., M.W. Vasconcelos and M.A. Grusak. 2007. Expression profiling of *Oryza sativa* metal homeostasis genes in different rice cultivars using a cDNA macroarray. Plant Physiology and Biochemistry 45(5): 277-286.
24. Ohdan, T., P.B.J. Francisco and T. Sawada 2005. Expression profiling of genes involved in starch synthesis in sink and source organs of rice. Journal of Experimental Botany 56(422): 3329-3244.
25. Ragland, M. and K.M. Soliman. 1997. Sali5-4a and Sali3-2: two genes induced by Al in soybean roots. Plant Physiology 114(1): 395-395.
26. Richards, K.D., K.C. Snowden and R.C. Gardner. 1994. Wali6 and wali7. Genes induced by aluminum in wheat (*Triticum aestivum* L.) roots. Plant Physiology 105(4): 1455-1456.
27. Salekdeh, G.H., J. Siopongco, L.J. Wade, B. Ghareyazie and J. Bennett. 2002. Proteomic analysis of rice leaves during drought stress

and recovery. Proteomics 2(9): 1131–1145.

28. Yin, L., J. Mano, S. Wang, W. Tsuji and K. Tanaka. 2009. The involvement of lipid peroxide-derived aldehydes in aluminum toxicity of tobacco roots. Plant Physiology 152(3): 1406–1417.

29. Zhen, Y., J.L. Qi, S.S. Wang, J. Su, G.H. Xu, M.S. Zhang et al. 2007. Comparative proteome analysis of differentially expressed proteins induced by Al toxicity in soybean. Physiologia Plantarum 131(4): 542–554.