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關鍵字(中)	菊花 HVA1 基因 LEA 蛋白質 鹽逆境 農桿菌轉殖 Real-time PCR
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摘要(中)	分離自以離素(abscisic acid, ABA)處理的大麥（Hordeum vulgare L.）粉糊層(aleurone layer)的 HVA1 蛋白，屬於第三類的 LEA(Late Embryogenesis Abundant)蛋白質，大小約為 22kDa。研究發現，自土壤吸水三天的植物幼苗，在離素、乾旱、冷害、鹽害及高溫等逆境處裡下，能夠迅速的表現出 HVA1 蛋白質。水稻為最早利用 HVA1 轉殖的植物，HVA1 基因轉

	殖水稻發現較能適應高鹽的逆境。到目前為止，已有許多 HVA1 基因轉殖作物被發表，例如：菸草(tobacco)、蕃茄(tomato)、小麥(wheat)及燕麥(oat)等，多為糧食作物或模式，尚無 HVA1 轉殖花卉的問世。因此本實驗利用農桿菌轉殖菊花，得到 HVA1 基因轉殖菊花，並比較轉殖株與栽培種菊花在鹽逆境下之抗氧化基因表現與生理型態之差異。經農桿菌轉殖後，獲得多株再生成功的菊花幼苗，選取具有抗生素抗性之擬轉殖菊花，經過 PCR 檢測確認獲得數個轉殖株。將切離葉片以鹽逆境處理，經 RT real-time PCR 結果顯示，以轉殖株 400mM NaCl 處理十分鐘後，catalase 基因即被誘導表現。而且 catalase 基因不但有週期性表現之現象，而且表現量逐漸增加。比較鹽逆境處理前後 catalase 基因的表現量，發現栽培種菊花之 catalase 基因表現量下降 98%，而轉殖株之 catalase 基因表現量，最低下降 92%，最高則上升至 214%。在 catalase 活性表現方面，轉殖株的 catalase 活性增加量均比栽培種高，栽培種之 catalase 活性增加量僅 0.29，轉殖株的 catalase 活性增加量在 1.63 至 4.31 之間。由此得知，我們所獲得之 HVA1 基因轉殖菊花在鹽逆境下，其 catalase 基因的表現程度較野生株強，具較佳之抗氧化活性。
摘要(英)	The barley HVA1 is belong to group 3 LEA (late embryogenesis abundant) protein and its molecular weight is about 22kDa. The HVA1 protein was firstly isolated from the aleurone layer of the barley seed. HVA1 mRNA was rapidly induced in young seedlings (3 days after imbibition) by ABA treatment and a series of stress conditions, such as drought, cold, NaCl, and heat. The HVA1 transgenic rice harboring HVA1 gene was found to be more tolerant to the salt stress than wild-type. In order to increase the salt tolerance of chrysanthemum, we try to produce transgenic chrysanthemum by Agrobacterium-mediated transformation. After Agrobacterium transformation, we have obtained a lot of chrysanthemum seedlings. By putting detached leaf of chrysanthemum seedling in a selection medium, we acquired several chrysanthemum plants which may contain HVA1 gene. These putative transgenic plants were confirmed by PCR analysis. By the analysis of RT real-time PCR, the expression of catalase gene of transgenic plant was found to induce when the detached leaf put in salt stress for 10 min. Morever, we find that catalase gene is regulated by a circadian rhythm, and the expression of catalase increases with every rhythm cycle. Compared with the extent of catalase expression before and after salt stress treatment, the catalase of wild-type reduces 98%, while transgenic plants are from -92% to 214%. Detecting the catalase activity of detached leaf treated with salt stress or water, we find that the increase of catalase activity in transgenic plant is higher than that in wild-type plant. The catalase cativity only increase 0.29 in wild-type, however, the catalase cativity increase from 1.63 to 4.31 in transgenic plants. We conclude that the transgenic chrysanthemum plants containing HVA1 have higher antioxidant ability under salt stress.
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王昆、王穎、鮑永利、孟祥穎、烏垠、李玉新。(2006)。提取高質量人參 RNA 方法 研究。生物化學與生物物理發展 33，95-99。 張錦興、張元聰、王仕賢。(2000)。雲嘉南地區菊花產業簡介。台南區農業專訊 32，

13-19。 Alary, R., Serin, A., Duviau, M.P., Joudrier, P., and Gautier, M.F. (2002). Quantification of common wheat adulteration of durum wheat pasta using real-time quantitative polymerase chain reaction (PCR). Cereal Chem 79, 553-558. Aljanabi, S.M., Forget, L., and Dookun, A. (1999). An Improved and Rapid Protocol for the Isolation of Polysaccharide- and Polyphenol-Free Sugarcane DNA. Plant Mol Biol Rep 17, 1-8. Babu, C.R., Zhang, J., Blum, A., Ho, T.H., Wu, R., and Nguyen, H.T. (2004). HVA1, a LEA gene from barley confers dehydration tolerance in transgenic rice (Oryza sativa L.) via cell membrane protection. Plant Sci 166, 855-862. Bahieldin, A., Mahfouz, H.T., Eissa, H.F., Saleh, O.M., Ramadan, A.M., Ahmed, I.A., Dyer, W.E., El-Itriby, H.A., and Madkour, M.A. (2005). Field evaluation of transgenic wheat plants stably expressing the HVA1 gene for drought tolerance. Physiol Plant 123, 421-427. Baker, J., Steele, C., and Dure III, L. (1988). Sequence and characterization of 6 LEA proteins and their genes from cotton. Plant Mol Biol 11, 277-291. Bohm, J., Hahn, A., Schubert, R., Bahnweg, G., Adler, N., Nechwatal, J., Oehlmann, R., and Osswald, W. (1999). Real-time quantitative PCR: DNA determination in isolated spores of the mycorrhizal fungus Glomus mossae and monitoring of Phytophthora infestans and Phytophthora citricola in their respective host plants. J Phytopathol 147, 409-416. Bray, E.A. (1993). Molecular responses to water deficit. Plant Physiol 103, 1035-1040. Bray, E.A., Bailey-Serres, J., and Weretinyk, E. (2000). Responses to abiotic stresses. Biochemistry and molecular biology of plants. American Society of Plant Physiologists, Rockville, MD, 1158-1249. Bush, A.L., and Pueppke, S.G. (1991a). Characterization of an unusual new Agrobacterium tumefaciens strain from Chrysanthemum morifolium Ram. Appl Environ Microbiol 57, 2468-2472. Bush, A.L., and Pueppke, S.G. (1991b). Cultivar-strain specifity between chrysanthemum morifolium and Agrobacterium tumefaciens. Plant Mol Biol Pathol 39, 309-323. Chaves, M.M., Marco, J.P., and Pereira, J.S. (2003). Understanding plant responses to drought-from genes to the whole plant. Funct Plant Biol 30, 239-264. Chen, Y., Qu, Y.Q., and Jia, X. (2003). The characters and gene expression of rice seed proteins. Hereditas 25, 367-372. Chen, Z.Q., Liu, Q., Zhu, Y.S., and Li, Y.X. (2002). Assessment on predicting methods for membrane-protein transmembrane regions. Acta Biochem Biophys Sin 34, 285-290. Close, T.J., Kortt, A.A., and Chandler, P.M. (1989). A cDNA-based comparison of dehydration-induced proteins (dehydrins) in barley and corn. Plant Mol Biol 13, 95-108. Courtney-Gutterson, N., Napoli, C., Lemieux, C., Morgan, A., Firoozabady, E., and Robinson, K. (1994). Modification of lower color in florist chrysanthemum: production of a white-flowering variety through molecular genetics. Biotechnol 12, 268-271. CPRO-DLO. (1997). Ornamental crops. Annual Report, 13. Cuming, A.C. (1999). LEA proteins. In Seed Proteins. (Kluwer Academic). Curry, J., and Walker-Simmons, M.K. (1993). Unusual sequence of group 3 LEA (II) mRNA inducible by dehydration stress in wheat. Plant Mol Biol 21, 907-912. Curry, J., Morris, C.F., and Walker-Simmons, M.K. (1991). Sequence analysis of a cDNA encoding a group 3 LEA mRNA inducible by ABA or dehydration stress in wheat. Plant Mol Biol 16, 1073-1076. De Jong, J., Randemaker, W., and van Wordragen,

M.F. (1993). Restoring adventitious shoot formation on Chrysanthemum leaf explants following cocultivation with Agrobacterium tumefacians. Plant Cell Tissue Organ Cul. 32, 263-270. Dure, I.L., and Crouch, M. (1981). Developmental biochemistry of cotton seed embryogenesis, and termination: changing messenger ribonucleic and populations as shown by in vitro and in vivo protein synthesis. Biochem 20, 4162-4168. Gachon, C., Mingam, A., and Charrier, B. (2004). Real-time PCR: what relevance to plant studies? J Exp Bot 55, 1445-1454. Goyal, K., Walton, L.J., and Tunnacliffe, A. (2005). LEA proteins prevent protein aggregation due to water stress. Biochem J 388, 151-157. Han, B. (2003). Plant functional genome research. Bull Chin Acad Sci 4, 250-254. Han, B., and Kermode, A.R. (1996). Dehydrin-like proteins in castor bean seeds and seedling are differentially produced in response to ABA and water-deficit-related stress. J Exp Bot 42, 933-939. Hare, P.D., Cress, W.A., and Van Staden, J. (1998). Dissecting the roles of osmolyte accumulation during stress. Plant Cell Environ 21, 535-553. He, J.X., and Fu, J.R. (1996). The research progresses in Lea proteins of seeds. Plant Physiol Commun 32, 241-246. Hohne, M., Santisi, C.R., and Meyer, R. (2002). Real-time multiplex PCR: an accurate method for the detection and quantification of 35SCaMV promoter in genetically modified maize-containing food. European Food Research and Technology 215, 59-64. Hong, B., Uknes, S.J., and Ho, T.H. (1988). Cloning and characterization of cDNA encoding a mRNA repidly-induced by ABA in barley aleurone layers. Plant Mol Biol 11, 495-506. Hong, B., Brag, R., and Ho, T.H. (1992). Developmental and organ-specific expression of an ABA- and stress-induced protein in barley. Plant Mol Biol 18, 663-674. Hossain, Z., Azad Mondal, A.K., Datta, S.K., and Biswas, A.K. (2006). Isolation of a NaCl-tolerant mutant of Chrysanthemum morifolium by gamma radiation: in vitro mutagenesis and selection by salt stress. Funct Plant Biol 33, 91-101. Huggett, J., Dheda, K., Bustin, S., and Zumla, A. (2005). Real-time RT-PCR normalisation; strategies and considerations. Genes and Immunity 6, 279-284. Jeong, J.H., Chakrabarty, D., Kim, S.J., and Paek, K.Y. (2002). Transformation of chrysanthemum (Dendranthema grandiflorum Kitamura cv Cheonsu by constitutive expression of rice OsMADS1 gene. J Kor Soc Hortic Sci 43, 382-386. Kato, M., and Shimizu, S. (1987). Chlorophyll metabolism in higher plants. VII. Chlorophyll degradation in senescing tobacco leaves; phenolic-dependent peroxidase degradation. Can J Bot 65, 729-735. Kishor, P.B.K., Hong, Z., Miao, G.H., Hu, C.A.A., and Verma, D.P.S. (1995). Overexpression of △1-pyrroline-5-carboxylate synthetase increses proline production and confers osmotolerance in transgenic plants. Plant Physiol 108, 1387-1394. Ledger, S.E., Deroles, S.C., and Given, N.K. (1991). Regeneration and Agrobacterium-mediated transformation of Chrysanthemum. Plant Cell Rep. 10, 195-199. Li, N.Y., Gao, J.F., and Wang, P.H. (1998). Protein characters induced by water stresses in wheat seedlings. Acta Phytophysiol 24, 65-71. Luna, C.M., Pastori, G.M., Driscoll, S., Groten, K., Bernard, S., and Foyer, C.H. (2004). Drought controls on H2O2 accumulation, catalase (CAT) activity and CAT gene expression in wheat. J Exp Bot 56, 417-423. McCubbin, W.D., Kay, C.M., and Lane, B.G. (1985). Hydrodynamic and optical

properties of wheat germ Em protein. Can J Biochem Cell Biol 63, 803-811. McKersie, S.D., Bowley, S.R., Harjanto, E., and Leprince, O. (1996). Water deficit tolerance and field performance of transgenic alfalfa overexpressing superoxide dismutase. Plant Physiol 111, 1177-1181. Mitra, J. (2001). Genetics and genetic improvement of drought resistance in crop plants. Curr Sci 80, 758-763. Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7, 405-410. Ogawa, Y., Ishikawa, K., and Mii, M. (2000). Highly tumorigenic Agrobacterium tumefaciens strain from crown gall tumors of chrysanthemum. Arch Microbiol 173, 311-315. Oraby, H.F., Ransom, C.B., Kravchenko, A.N., and Sticklen, M.B. (2005). Barley HVA1 Gene Confers Salt Tolerance in R3 Transgenic Oat. Crop Sci 45, 2218-2227. Petty, L.M., Harberd, N.P., Carre?, I.A., Thomas, B., and Jackson, S.D. (2003). Expression of the Arabidopsis gai gene under its own promoter causes a reduction in plant height in chrysanthemum by attenuation of the gibberellin response. Plant Sci 164, 175-182. Pilon-Smits, E.A.H., Ebskamp, M.J.M., Paul, M.J., Jeuken, M.J.W., Weisbeek, P.J., and Smeekens, S.C.M. (1995). Improved performance of transgenic fructan-accumulating tobacco under drought stress. Plant Physiol 107, 125-130. Redinbaugh, M.G., Sabre, M., and Scandalios, J.G. (1990). Expression of the maize Cat3 catalase gene is under the influence of a circadian rhythm. Proc Natl Acad Sci USA 87, 6853-6857. Renou, J.P., Brochard, P., and Jalouzot, R. (1993). Recovery of transgenic chrysanthemum (Dendranthema grandiflora Tzvelev) after hygromycin resistance selection. Plant Sci 89, 185-197. Rizhsky, L., Liang, H.J., and Mittler, R. (2002). The combined effect of drought stress and heat shock on gene expression in tobacco. Plant Physiol 130, 1455-1463. Roberts, J.K., DeSimone, N.A., Lingle, W.L., and Dure III, L. (1993). Cellular concentrations and uniformity of cell-type accumulation of two LEA proteins in cotton embryos. Plant Cell 5, 769-780. Robinson, K.E.P., and Firoozabady, E. (1993). Transformation of floriculture crops. Sci Hortic 55. Roxas, V.P., Smith, R.K.J., Allen, E.R., and Allen, R.D. (1997). Overexpression of glutathione S-transferase/glutathione peroxidase enhances the growth of transgenic tobacco seedling during stress. Nature Biotech 15, 988-991. Scoltis, D.E., and Soltis, P. (2003). The role of phylogenetic in comparative genetics. Plant Physiol 132, 1790-1800. Seffens, W.S., Almoguera, C., Wilde, H.D., Vonder-Haar, R.A., and Thomas, T.L. (1990). Molecular analysis of a phylogenetically conserved carrot gene: developmental and environmental regulation. Dev Genet 11, 65-76. Serrano, R., Mulet, J.M., Rios, G., Marquez, J.A., de Larrinoa, I.F., Leube, M.P., Mendizabal, I., Pascual-Ahuir, A., Proft, M., Ros, R., and Montesinos, C. (1999). A glimpse of the mechanisms of ion homeostasis during salt stress. J Exp Bot 50, 1023-1036. Shao, H.-B., Liang, Z.-S., and Shao, M.-A. (2005). LEA proteins in higher plants: Structure, function, gene expression and regulation. Colloids Surf B Biointerfaces 45, 131-135. Sivamani, E., Bahieldin, A., Wraith, J.M., Al-Niemi, T., Dyer, W.E., Ho, T.H., and Qu, R. (2000). Improved biomass productivity and water use efficiency under water deficit conditions in transgenic wheat constitutively expressing the barley HVA1 gene. Plant Sci 155, 1-9. Snyder, M.,

and Gerstein, M. (2003). Genomics. Defining genes in the genomics era. Science 300, 258-260. Swire-Clark, G.A., and Marcotte, W.J. (1999). The wheat LEA protein Em functions as an osmoprotective molecule in Saccharomyces cerevisiae. Plant Mol Biol 39, 117-128. Takatsu, Y., Tomotsune, H., Kasumi, M., and Sakuma, F. (1998). Differences in adventitious shoot regeneration capacity among Japanese chrysanthemum (Dendranthema grandiflora (Ramat.) Kitamura) cultivars and the improved protocol for Agrobacterium-mediated genetic transformation. J Jpn Soc Hortic Sci 67, 958-964. Tarczynski, M.C., Jensen, R.G., and Bohnert, H.J. (1993). Stress protection of transgenic tobacco by production of osmolyte mannitol. Science 259, 508-510. Teixeira da Silva, J.A., and Fukai, S. (2002a). Polyamines in the regulation of chrysanthemum and tobacco in vitro morphogenic pathways. Prop Ornamental Plants 2, 9-15. Teixeira da Silva, J.A., and Fukai, S. (2002b). Change in transgene expression following transformation of chrysanthemum by four gene introduction methods. Prop Ornamental Plants 2, 28-37. Texieira da Silva, J. (2003). Chrysanthemum: advances in tissue culture, cryopreservation, postharvest technology, genetics and transgenic biotechnology. Biotechnol Adv. 21, 715-766. Thomashow, M.F. (1998). Role of cold-responsive genes in plant freezing tolerance. Plant Physiol 118, 1-7. Thomashow, M.F. (1999). Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. Annu Rev Plant Biol 50, 571-599. Trick, H.N., and Finer, J.J. (1997). Sonication-assisted Agrobacterium-mediated transformation. Transgenic Res 6, 329-337. Urban, L.A., Sherman, J.M., Moyer, J.W., and Daub, M.E. (1994). High frequency shoot regeneration and Agrabacterium-mediated transformation of chrysanthemum (Dendranthema grandiflora). Plant Sci 98, 69-79. Wang, W., Vinocur, B., and Altman, A. (2003). Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta 218, 1-14. Welin, B.V. (1994). Characterization and differential expression of dhn / lea / rab-like genes during cold acclimation and drought stress in Arabidopsis thaliana. Plant Mol Biol 26, 131-144. Weller, S.A., Elphinstone, J.G., Smith, N.C., Boonham, N., and Stead, D.E. (2000). Detection of Ralstonia solanacearum strains with a quantitative, multiplex, real- time, fluorogenic PCR (TaqMan) assay. Applied Environ Microbio 66, 2853-2858. Wise, M.J. (2003). LEAping to conclusions: a computational reanalysis of late embryogenesis abundant proteins and their possible roles. BMC Bioinform 4, 52. Wisniewski, K., and Zagdanska, B. (2001). Genotype-depedent proteolytic response of spring wheat to water deficiency. J Exp Bot 52, 1455-1463. Xu, D., Duan, X., Wang, B., Hong, B., Ho, T.H., and Wu, R. (1996). Expression of a late embryogenesis abundant proteitn gene, HVA1, from barley confers tolerance to water deficit and salt stress in transgenic rice. Plant Physiol 110, 249-257. Yepes, L.C., Mittak, V., Pang, S.Z., Gonsalves, C., Slightom, J.L., and Gonsalves, D. (1995). Agrobacterium tumefaciens versus biolistic-mediated transformation of the chrysanthemum cvs Polaris and Golden Polaris with nucleocapsid protein genes of three Tospovirus species. Acta Hortic 482, 209-218. Yu, J.N., Zhang, L.S., and Gao, J.F. (2002). Effect of water stress on induced protein expression of wheat seedlings and auapension cultures. Acta

	Biochem Biophys Sin 52, 865-870. Zhang, H.X., Hodson, J.N., Williams, J.P., and Blumwald, E. (2001). Engineering salt-tolerant Brassica plants: characterization of yield and seed oil quality in transgenic plants with increased vacuolar sodium accumulation. Proc Natl Acad Sci USA 98, 12832-12836. Zhang, J.F., Deng, X.P., and Mu, X.Q. (2002). Plant aquaporin. Plant Physiol Commun 38, 88-91. Zhang, L.S., and Zhao, W.M. (2003). LEA protein functions to tolerance drought of the plant. Plant Physiol Commun 39, 61-66. Zheng, Z.L., Yang, Z., Jang, J.C., and Metzger, J.D. (2001). Modification of plant architecture in chrysanthemum by ectopic expression of the tobacco phytochrome B1 gene. J Am Soc Hortic Sci 126, 19-26. Zhu, J.K. (2001). Plant salt tolerance. Trends Plant Sci 6, 66-71.
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