利用功能性互補法篩選阿拉伯芥氧化逆境訊號相關因子

葉怡芳、洪淑嫻

E-mail: 9501063@mail.dyu.edu.tw

摘 要

本研究以阿拉伯芥為材料，利用酵母菌Yap1基因剔除突變株，以功能性互補法篩選阿拉伯芥氧化逆境訊號相關因子，探討植物對過氧化氫訊號之回應。為了增進篩選效率，將酵母菌突變株與野生株以不同比例混合，模擬多菌株集合培養方式進行初步篩選，根據此模擬狀況，已建立了快速篩選系統，藉由此系統，篩選到一株具過氧化氫抗性突變株並證實為酵母菌Yap1基因缺陷突變株發生突變所造成。此外，也對過氧化氫所誘導阿拉伯芥的抗凍能力進行生理性的探討。阿拉伯芥植株前處理過氧化氫，可誘導植株獲得與冷馴化 (cold acclimation) 相當之抗凍能力。但植株經鈣離子螯合劑EGTA、鈣離子通道抑制劑 La3+或蛋白質激? (calcium dependent protein kinase) 抑制劑W7前處理，則過氧化氫所誘導之抗凍能力明顯受到抑制。因而推論過氧化氫所誘導之植株抗凍能力，需透過鈣離子及蛋白質激? “ @為訊號傳遞者，以啟動下游抗低溫基因的表現，增進植株抗凍能力。

關鍵詞 : 阿拉伯芥 ; 過氧化氫 ; Yap1 ; 抗凍能力

目錄

參考文獻

1. 李國維。1999。阿拉伯芥超氧歧化?調控之研究。國立台灣大學物研究所碩士論文。台灣，台北。 2. Allan, A.C., and Fluhr, R. 1997. Two district sources of elicited reactive oxygen species in tobacco epidermal cells. Plant Cell 9: 1559-1572. 3. Alvarez, M.E., Pennell, R.I., Meijer, P.J., Ishikawa, A., Dixon, R.A. and Lamb, C. 1998. Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity. Cell 92: 773-784. 4. Alvarez, S. M., Rufenacht, K., and Eggen R. L. 2000. The oxidative stress-sensitive yap1 null strain of Saccharomyces cerevisiae becomes resistant due to increased carotenoid levels upon the introduction for the 60S ribosomal protein L10a. Biochem. and Biophl. Res. Commun. 267: 953-959. 5. Ashworth, E. N. 1989. Freezing injury in deciduous fruit crops: opportunities for chemical manipulation. Acta Hort. 239: 175-186. 6. Azevedo, D., Tacnet, F., Delaunay, A., Pousada, C. R., and Toledano, M.B. 2003. Two redox centers within YAP1 for H2O2 And thiol-reactive chemicals signaling. Free Radic. Biolo. Med. 35: 889- 900. 7. Belles-Boix, E., Babiychuk, E., Montagu, M. V., Inze, D., and Kushnir, S. 2000. CEO1, a new protein from Arabidopsis thaliana, protects yeast against oxidative damage. FEBS Lett. 482: 19-24. 8. Belles-Boix, E., Babiychuk, E., Montagu, M. V., Inze, D., and Kushnir, S. 2000. CEF, a Sec24 homologue of Arabidopsis thaliana, enhances the survival of yeast under oxidative stress conditions. J. Exp. Bot. 51: 1761-1762. 9. Bowler, C., Marc, VM.,Dirk, I. 1992. Superoxide dismutase and stress tolerance. Annw. Rev. Plant Physol. Plant Mol. Biol. 43: 83-116. 10. Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram Quan ties of protein utilizing the principle of protein-dye bindings. Anal. Biochem. 72: 248-254.Browse, J., Xin, Z. 2001. Temperature sensing and cold acclimation. Curr. Opin. Plant Bio. 4: 241-246. 11. Browse, J., Xin, Z. 2001. Temperature sensing and cold acclimation. Curr. Opin. Plant Bio 4: 241-246. 12. Cohen, B. A., Pilpel, Y., Mitra, R. D., and Church, G. M. 2002. Discrimination between paralogs using microarray analysis : application to the Yap1p and Yap2p transcriptional networks. Mol. Biol.Cell 13: 1608-1614. 13. Coleman, S. T., Epping, E. A., Steggerda, S. M., and Moye Rowley,W.S. 1999. Yap1p activates gene transcription in an oxidant specific fashion. Mol. Cell. Biol. 19: 8302-8313. 14. Delaunay, A., Isnard, A. D., and Toledano, M. B. 2000. H2O2 sensing through oxidation of the Yap1 transcription factor. EMBO J. 19: 5157-5166. 15. Gidrol, X., Sabelli, P. A., Fern, Y. S., and Kush, A. K. 1996. Annexin-like protein from Arabidopsis thaliana rescues oxyR mutant of Escherichia coli from H2O2 stress. Proc. Natl. Acad. Sci. USA 93: 11268-11273. 16. Gong, M., van der Luit, A. H, Knight, M. R ,and Trewavas, A. J. 1998. Heat shock induced changes in intracellular Ca2+ level in tobacco seedling in relation to thermotolerance. Plant Physiol. 116: 429-437. 17. Grant, C. M., Collinson, L. P., Roe, J-H., and Dawes, I. W. 1996. Yeast glutathione reductase is required for protection against oxidative stress and is a target gene for YAP-1 transcriptional requlation. Mol. Microbiol. 21: 171-179. 18. Hariyadi, P., and Parkin, K.L. 1993. Chilling-induced oxidative stress in cucumber (Cucumis sativus L. cv. Calypso) seedlings. J. Plant Physiol. 141: 733-738. 19. Inoue, Y., Matsuda, T., Sugiyama, K., Izawa, S., and Kimura, A. 1999. Genetic analysis of glutathione peroxidase in oxidative stress reponse of Saccharomyces cerevisiae. J Biol Chem 274: 27002-27009. 20. Jena, S and Choudhuri, MA. 1981. Glycolate metabolism of three submerged aquatic angiosperms during aging. Aquat. Bot. 12: 345-354. 21. Kendall, A. C., Keys, A. J., Turner, J. C., Lea, P. J., Miflin, B. J. 1983. The isolation and characterization of a catalase-deficient mutant of barley (Horfeum vulgare L). Planta 159: 505-511. 22. Knight, H., Trewavas, A. J., and Knight, M. R. 1996. Cold calcium signaling in Arabidopsis involves two cellular pools and a change in calcium signature after acclimation. Plant Cell 8: 489-503. 23. Larkindale, J., and Knight, M. R. 2002. Protection against heat stress induced oxidative damage in Arabidopsis involves calcium, abscisic acid, ethylene, and salicylic acid. Plant Physiol. 128: 682-695. 24. Levitt, J. 1980. Response of plants to plants to elvironmental stress. Vol. 1. Chilling, freezing and high temperature stresses. 2nd ed. New York, Academic Press. 25. Liu, H. T., Li, B., Shang, Z. L., Li, X. Z., Mu, R. L., Sun, D. Y., and Zhou, R.G. 2003. Calmodulin is involved in heat shock signal transducation in wheat. Plant Physiol. 132: 1186-1195. 26. Monroy, A. F., Fathey Sarhan, and Dhindsa, R. S. 1993. Cold induced changes in freezing tolerance, protein phosphorylation, and gene expression. Plant Physiol. 102: 1227-1235. 27. Monroy, A. F., and Dhindsa, R. S. 1995. Low-temperature signal transduction: induction of cold acclimation-specific genes of Alfalfa by calicium at 25oC. Plant Cell 7: 321-331. 28. Morgan, B. A., Banks, G. R., Toone, W. M., Raitt, D., Kuge, S., and Johnston, L. H. 1997. The Skn7 respose regulator control gene expression in the oxidative stress response of budding yeast Saccharomyces cerevisiae. EMBO J. 16: 1035-1044. 29. Moye-Rowley, W. S., Harshma, K. D., and Parker, C. S. 1989. Yeast YAP1 encodes a novel form of the jun family of transcriptional activator proteins. Genes Dev. 3: 283-292. 30. Neuenschwander, U., Vernooij, B., Friedrich, L., Uknes, S., Kessmann, H., and Ryals, J. 1995. Is hydrogen peroxide a second messenger of salicylic acid in systemic acquired resistance? Plant J. 8: 227-233. 31. O ’ Kane, D., Gill, V., Boyd, P., and Burdon, R. 1996. Chilling, oxidative stress and antioxidant responses in Arabidopsis thaliana callus. Planta 198: 371-377. 32. Okuda, T., Matsuda, Y., Yamanaka, A., and Sagisaka, S. 1991. Abrupt increase in the level of hydrogen peroxide in leaves of winter wheat is caused by cold treatment. Plant Physiol. 97: 1265-1267. 33. Orozco-Cardenas, M., Narvaez-Vasquez, J., and Ryan, C. 2001. Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to wounding, systemin, and methyl jasmonate. Plant Cell 13: 179-191. 34. Palate J. P., Levitt J., Stadelman E. J. 1977. Freezing injury in onion bulb cells. I. Evaluation of the conductivity method and analysis of ion and sugar efflux from injured cells. Plant Physiol. 60: 393-397. 35. Potikha, T. S., Collins, C. C., Johnson, D. I., Delmer, D. P., and Levine,A, 1999. The involvement of hydrogen peroxide in the Differentiation of secondary walls in cotton fibers. Plant Physiol. 119: 849-858. 36. Prasad, T. K., Anderson, M. D., Martin, B. A., and Stewart, C. R. 1994. Evidence for chilling induced oxidative stress in maize seddling and a regulatory role for hydrogen peroxide. Plant Cell 6: 65-74. 37. Prasad, T.K. 1996. Mechanisms of chilling-induced oxidative stress injury and tolerance in developing maize seedlings: changes in antioxidant system, oxidation of proteins and lipids, and protease activities. Plant J. 10: 1017-1026. 38. Reichheld, J.P., Vernoux, T., Lardon, F., Montagu, M. V. , and Inze, D. 1999. Specific checkpoints regulate plant cell cycle

progression in response to oxidative stress. Plant J. 17: 647-656. 39. Ren, D., Yang, H., and Zhang, S. 2002. Cell death mediated by MAPK is associated with hydrogen peroxide production in Arabidopsis. J.Biol. Chem. 277: 559-565. 40. Rentel, M. C., and Knight, M. R. 2004. Oxidative stress induced calcium signaling in Arabidopsis. Plant Physiol. 135: 1471-1479 41. Sambrook J, and Russell DW. 2001. Molecular Cloning: a laboratory manual, third edition. Cold Spring Harbor Laboratory Press, New York. 42. Tahtiharju, S., Sangwan, V., Monroy, A. F., Dhindsa, R. S., Borg, M. 1997. The induction of Kin genes in cold acclimating Arabidopsis thaliana. Evidence of a role for calcium. Planta 203: 442-447. 43. Uchida, A., Jagendorf, A. T., Hibino, T., Takabe, T., and Takabe. T. 2002. Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. Plant Sci. 163: 515-523. 44. Wanner, L. A and Junttila, O. 1999. Cold induced freezing tolerance in Arabidopsis. Plant Physiol. 120: 391-399. 45. White, P.J., Bowen, H. C., Demidchik, V., Nichols, C., Davies, J. M.2002. Genes for calcium permeable channels in the plasma membrane of plant root cells. Biochim. Biophys. Acta 1564: 299-309. 46. Wingsle, G. and Hallgren, J.E. 1993. Influence of SO2 and NO2 exposure on glutathione, superoxide dismutase and glutathione reductase activities in Scots pine needles. J. Exp. Bot. 44: 463-470. 48. Yu, C. W., Murphy T. M., Sung, W. W, and Lin C. H. 2002. H2O2 treatment induces glutathione accumulation and chilling tolerance in mung bean. Funct. Plant Biol. 29: 1081-1087. 49. Yu, C. W., Murphy T. M., and Lin C. H. 2003. Hydrogen peroxide induced chilling tolerance in mung beans mediated through ABA idependent glutathione accumulation. Funct. Plant Biol. 30: 995-963. 50. Zhang. L., Onda. K., Imai. R., and Fukuda. R. 2003. Growth temperature downshift induces antioxidant response in Saccharomyces cerevisiae. Biochem and Biophl Res Commun. 307: 308-314.