Increased tolerance to cold stress in transgenic Arabidopsis Thaliana expressing glucose oxidase of Aspergillus Niger /

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ABSTRACT

Hydrogen peroxide (H2O2) is thought as the signaling factor of plants under stress in a number of regulatory mechanisms to improve stress tolerance. In general, extreme temperature often adversely affects crop productivity resulting in the shortage of human food supply. However, it is still unclear the endogenous hydrogen peroxide would also improve the cold-tolerance of plant. In order to understand the role of hydrogen peroxide in plant under low temperature stress, and the molecular machanism to increase plant cold-resistant performance, the transgenic plants were constructed that glucose oxidase (GO) gene was under the control of cauliflower mosaic virus (CaMV) 35S constitutive promoter and glucose oxidase could activate endogenous hydrogen peroxide production. The cold-tolerance of the transgenic plants was investigated in comparison with the wild type plants for survival and leakage rates. The results suggested that transgenic plants exhibit enhanced cold-tolerance. Based on relative genes expression study the molecular mechanism was also discussed.

Keywords: glucose oxidase, cold stress, hydrogen peroxide, Arabidopsis thaliana

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REFERENCES

參考文獻 1. Wang W, Vinocur B, Altman A. Plant responses to drought, salinity and extreme temperatures: Towards genetic engineering for stress tolerance. Planta. 2003;218:1-14 2. Sanghera GS, Wani SH, Hussain W, Singh NB. Engineering cold stress tolerance in crop plants. Curr Genomics. 2011;12:30-43 3. Smallwood M, Bowles DJ. Plants in a cold climate. Philos Trans R Soc Lond B Biol Sci. 2002;357:831-847 4. Chinnusamy V, Zhu J, Zhu JK. Cold stress regulation of gene expression in plants. Trends Plant Sci. 2007;12:444-451 5. Desikan R, S AH-M, Hancock JT, Neill SJ. Regulation of the arabidopsis transcriptome by oxidative stress. Plant Physiol. 2001;127:159-172 6. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature. 2000;408:239-247 7. Slesak I, Libik M, Karpinska B, Karpinski S, Miszalski Z. The role of hydrogen peroxide in regulation of plant metabolism and cellular signalling in response to environmental stresses. Acta Biochim Pol. 2007;54:39-50 8. Prasad TK, Anderson MD, Martin BA, Stewart CR. Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide. Plant Cell. 1994;6:65-74 9. Neill S, Desikan R, Hancock J. Hydrogen peroxide signalling. Curr Opin Plant Biol. 2002;5:388-395 10. Mittler R, Vanderauwera S, Gollery M, Van Breusegem F. Reactive oxygen gene network of plants. Trends Plant Sci. 2004;9:490-498 11. Fahnenstich H, Scarpeci TE, Valle EM, Flugge UI, Maurino VG. Generation of hydrogen peroxide in chloroplasts of arabidopsis overexpressing glycolate -32- oxidase as an inducible system to study oxidative stress. Plant Physiol. 2008;148:719-729 12. Hung SH, Yu CW, Lin CH. Hydrogen peroxide functions as a stress signal in plants. Bot Bull Acad Sinica. 2005;46:1-10 13. Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 2002;7:405-410 14. Kwak JM, Nguyen V, Schroeder JI. The role of reactive oxygen species in hormonal responses. Plant Physiol. 2006;141:323-329 15. Gechev TS, Hille J. Hydrogen peroxide as a signal controlling plant programmed cell death. J Cell Biol. 2005;168:17-20 16. Neill SJ, Desikan R, Clarke A, Hurst RD, Hancock JT. Hydrogen peroxide and nitric oxide as signalling molecules in plants. J Exp Bot. 2002;53:1237-1247 17. Kachroo A, He Z, Patkar R, Zhu Q, Zhong J, Li D, Ronald P, Lamb C, Chattoo BB. Induction of H2O2 in transgenic rice leads to cell death and enhanced resistance to both bacterial and fungal pathogens. Transgenic Res.

2003;12:577-586 18. Mabilleau G, Bourdon S, Joly-Guillou ML, Filmon R, Basle MF, Chappard D. Influence of fluoride, hydrogen peroxide and lactic acid on the corrosion resistance of commercially pure titanium. Acta Biomater. 2006;2:121-129 19. Halliwell B. Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. Plant Physiol. 2006;141:312-322 20. Vranova E, Inze D, Van Breusegem F. Signal transduction during oxidative stress. J Exp Bot. 2002;53:1227-1236 21. Yu CW, Murphy TM, Sung WW, Lin CH. Hydrogen peroxide-induced chilling tolerance in mung beans mediated through ABA-independent glutathione accumulation. Funct Plant Biol 2003:30:955-963 22. Larkindale J, Huang B. Thermotolerance and antioxidant systems in agrostis stolonifera: Involvement of salicylic acid, abscisic acid, calcium, hydrogen -33- peroxide, and ethylene. J Plant Physiol. 2004;161:405-413 23. Wu G, Shortt BJ, Lawrence EB, Levine EB, Fitzsimmons KC, Shah DM. Disease resistance conferred by expression of a gene encoding H2O2-generating glucose oxidase in transgenic potato plants. Plant Cell. 1995;7:1357-1368 24. Wu G, Shortt BJ, Lawrence EB, Leon J, Fitzsimmons KC, Levine EB, Raskin I, Shah DM. Activation of host defense mechanisms by elevated production of H2O2 in transgenic plants. Plant Physiol. 1997;115:427-435 25. Lee JC, Son YO, Choi KC, Jang YS. Hydrogen peroxide induces apoptosis of bjab cells due to formation of hydroxyl radicals via intracellular iron-mediated fenton chemistry in glucose oxidase-mediated oxidative stress. Mol Cells. 2006;22:21-29 26. Lissarre M, Ohta M, Sato A, Miura K. Cold-responsive gene regulation during cold acclimation in plants. Plant Signal Behav. 2010;5:948-952 27. Chinnusamy V, Ohta M, Kanrar S, Lee BH, Hong X, Agarwal M, Zhu JK. Ice1: A regulator of cold-induced transcriptome and freezing tolerance in arabidopsis. Genes Dev. 2003;17:1043-1054 28. Wang Y, Hua J. A moderate decrease in temperature induces cor15a expression through the cbf signaling cascade and enhances freezing tolerance. Plant J. 2009;60:340-349 29. Novillo F, Medina J, Salinas J. Arabidopsis cbf1 and cbf3 have a different function than cbf2 in cold acclimation and define different gene classes in the cbf regulon. Proc Natl Acad Sci U S A. 2007;104:21002-21007 30. Stockinger EJ, Gilmour SJ, Thomashow MF. Arabidopsis thaliana cbf1 encodes an ap2 domain-containing transcriptional activator that binds to the c-repeat/dre, a cis-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit. Proc Natl Acad Sci U S A. 1997;94:1035-1040 31. Stitt M, Hurry V. A plant for all seasons: Alterations in photosynthetic carbon metabolism during cold acclimation in arabidopsis. Curr Opin Plant Biol. -34- 2002;5:199-206 32. Sakuma Y, Maruyama K, Osakabe Y, Qin F, Seki M, Shinozaki K, Yamaguchi-Shinozaki K. Functional analysis of an arabidopsis transcription factor, dreb2a, involved in drought-responsive gene expression. Plant Cell. 2006;18:1292-1309 33. Vainonen JP, Jaspers P, Wrzaczek M, Lamminmaki A, Reddy RA, Vaahtera L, Brosche M, Kangasjarvi J. Rcd1-dreb2a interaction in leaf senescence and stress responses in arabidopsis thaliana. Biochem J. 2011 34. Davletova S, Schlauch K, Coutu J, Mittler R. The zinc-finger protein zat12 plays a central role in reactive oxygen and abiotic stress signaling in arabidopsis. Plant Physiol. 2005;139:847-856 35. Rizhsky L, Davletova S, Liang H, Mittler R. The zinc finger protein zat12 is required for cytosolic ascorbate peroxidase 1 expression during oxidative stress in arabidopsis. J Biol Chem. 2004;279:11736-11743 36. Olson PD, Varner JE. Hydrogen peroxide and lignification. Plant J. 1993;4:887-892 37. Murray F, Llewellyn D, Mcfadden H, Last D, Dennis ES, Peacock WJ. Expression of the talaromyces flavus glucose oxidase gene in cotton and tobacco reduces fungal infection, but is also phytotoxic. Mol Breed. 1999;5:219-232 38. Orlova IV, Serebriiskaya TS, Popov V, Merkulova N, Nosov AM, Trunova TI, Tsydendambaev VD, Los DA. Transformation of tobacco with a gene for the thermophilic acyl-lipid desaturase enhances the chilling tolerance of plants. Plant Cell Physiol. 2003;44:447-450 39. WU Z, Chen LJ, Long YJ. Analysis of ultrastructure and reactive oxygen species of hyperhydric garlic (allium sativum I.) shoots. In Vitro Cell.Dev.Biol.-Plant. 2009;45:483-490 40. Nylander M, Svensson J, Palva ET, Welin BV. Stress-induced accumulation and tissue-specific localization of dehydrins in arabidopsis thaliana. Plant Mol Biol. -35- 2001:45:263-279 41. Maruthasalam S. Liu YL, Sun CM, Chen PY, Yu CW, Lee PF, Lin CH. Constitutive expression of a fungal glucose oxidase gene in transgenic tobacco confers chilling tolerance through the activation of antioxidative defence system. Plant Cell Rep. 2010;299:14. 42. Liu Y, Ye N, Liu R, Chen M, Zhang J. H2O2 mediates the regulation of aba catabolism and ga biosynthesis in arabidopsis seed dormancy and germination. J Exp Bot. 2010;61:2979-2990 43. Chen CC, Liang CS, Kao AL, Yang CC. Hhp1 is involved in osmotic stress sensitivity in arabidopsis. J Exp Bot. 2009;60:1589-16 44. 吳善鈞. 菸 草轉殖表現黑麴菌葡萄糖氧化?可提高對熱逆境之抗性. 大葉大學分子生物科技碩士論文. 2011