過氧化氫生成酵素glucose oxidase增進轉基因菸草抗病害能力之研究

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摘 要

Kazan等人研究指出轉殖真菌glucose oxidase基因至植物中，可使此植物提升抗病害的能力，但若採用持續性表現之起動子，將導致過量之過氧化氫生成而造成植物生長發育及繁殖方面之傷害。因此，本研究欲培養出具抗病性且生長正常之植株，以菸草(Nicotiana tabaccum L. cv. SR1) 為研究材料，轉殖真菌Aspergillus niger之glucose oxidase (GO; EC 1.1.3.4) 基因，透過誘導性起動子PR1-a promoter控制過氧化氫生成之時間。試驗證實，經水楊酸處理過之轉殖植株可誘導表現GO基因並促使細胞內過氧化氫含量提高。由病原接種試驗結果顯示，菸草轉殖外來GO基因確實能夠提升對水稻立枯絲核菌與白絹菌之抗性；藉由記錄其生長發育情形與種子數量，無論於生長高度、開花數、種子莢數等方面，野生型植物WT與轉殖株之間並無差別。3-3-2株系分析結果卻發現其發芽率、種子莢、種子總重量與種子重量等數值皆偏低，極可能係因其植株未經誘導就可產生較多過氧化氫所造成。綜合結果顯示，以誘導性起動子驅動GO基因，既能經誘導轉殖株植物細胞產生過氧化氫而增強抗病性，卻不會對植物造成傷害。

關鍵詞 : 病原菌、葡萄糖氧化?、過氧化氫、抗性、誘導性起動子、PR1-a起動子

目錄

參考文獻

1.李國欽。2004。合理化施肥。植物保護與永續農業:第1-7頁。2004年2月11日。當前永續經營研討會。中華永續農業協會。台中，台灣。 2.蔡竹固，編著。1995。植物病理學精要:第248-251頁，第272-273頁。台灣復文興業股份有限公司。台南，台灣。 3.吳善鈞。2011。菸草轉殖表現黑麴菌葡萄糖氧化?可提高對熱逆境之抗性:第17-21頁。大葉大學分子生物科技學研究所碩士班論文，彰化，台灣。 4.Agarwal, S., Sairam, R. K., Srivastava G. C., Tyagi A. and Meena R. C. 2005. Role of ABA, salicylic acid, calcium and hydrogen peroxide on antioxidant enzymes induction in wheat seedlings. Plant Sci., 169: 559-570. 5.Agrawal, G. K., Rakwal, R. and Jwa, N. 2000a. A novel rice (Oryza sativa L.) acidic PR1 gene highly responsive to cut phytohormones, and protein phosphatase inhibitors. Biochem. Biophys. Communic, 274: 157-165. 6.Agrawal, G. K., Rakwal, R. and Jwa, N. 2000b. Rice (Oryza sativa L.) OsPR1b gene is phytohormonally regulated in close interaction with light signals. Biochem. Biophys. Communic, 278: 290-298. 7.Agrios, G. N. 2005. Plant pathology. 5th edition. Elsevier Academic Press, Burlington, MA, USA. 8.Al-Barwani, F.M. and Eltayed, E.A. 2004. Antifungal compound from induced Conium maculatum L. plants. Biochemical Systematics and Ecology, 32: 1097-1108. 9.Alvarez, M. E. and Lamb, C. 1997. Oxidative burst-mediated defenseresponses in plant disease resistance. In: Scandalios JG (ed.), Oxidative Stress and the Molecular Biology of Antioxidant Defenses. 815-839. Cold Spring Harbor Laboratory, Plainview,NY, USA. 10.Alvarez, M. E., Pennell, R. I., Meijer, P. J., Ishikawa A., Dixon, R. A. and Lamb, C. J. 1998. Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity. Cell, 92: 773-784. 11.Barber, M. S., Bertram, R. E. and

Ride, J. P. 1989. Chitin oligosaccharides elicit lignification in wounded wheat leaves. Physiol. MOI. Plant Pathol., 34: 3-12. 12.Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem., 72: 248-254. 13.Bradley, D. J., Kjellbom, P. and Lamb, C. J. 1992. Elicitor- and wound induced oxidative cross-linking of a proline-rich plant cell wall protein: A novel, rapid defense response. Cell, 70: 21-30. 14.Bruce, R. J. and West, C. A. 1989. Elicitation of lignin biosynthesis and isoperoxidase activity by pectic fragments in suspension cultures of castor bean. Plant Physiol., 91: 889-897. 15.Chao, Y. Y. , Chen, C. T. , Huang, W. D. and Kao C. H. 2009. Salicylic acid-mediated hydrogen peroxide accumulation and protection against Cd toxicity in rice leaves. Plant Soil., 329: 327-337. 16.Chen, Z., Silva, H., and Klessig, D. F. 1993. Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. Science, 262: 1883-1886. 17.Dangl, J. L., and Jones, J. D. G. 2001. Plant pathogens and integrated defence responses to infection. Nature, 411: 826-833. 18.Dangl, J. L., Dietrich, R. A. and Richberg, M. H. 1996. Death don ' t have no mercy: cell death programs in plant-microbe interactions. Plant Cell, 8: 1793-1807. 19.Dat, J. F., Foyer, C. H. and Scott, I. M. 1998. Changes in salicylic acid and antioxidants during induced thermotolerance in mustard seedlings. Plant Physiol., 118: 1455-1461. 20.del Pozo, O. and Lam, E. 1998. Caspases and programmed cell death in the hypersensitive response of plants to pathogens. Curr. Biol., 8: 1129-1132. 21.Devlin, W. S. and Gustine, D. L. 1992. Involvement of the oxidative burst in phytoalexin accumulation and the hypersensitive reaction. Plant Physiol., 100: 1189-1195. 22.Dixon, D., Cutt J. and Klessig, D. 1991. Differential targeting of the tobacco. PR1EMBO J., 10: 1317-1324. 23.Dixon, R. A. and Lamb, C. J. 1990. Molecular communication in plant: microbial pathogen interactions. Ann. Rev. Plant Physiol.Plant Mol. Biol. 41: 339-367. 24.Dixon, R. A. and Harrison, M. J. 1990. Activation, structure and organization of genes involved in microbial defenses in plants. Adv. Genet, 28, 165-234. 25.Eckey-Kaltenbach, H., Ernst, D., Heller, W. and Sandermann, H. J. 1994. Biochemical plant responses to ozone. IV. Cross-induction of defensive pathways in parsley (Petroselinum crispum L.) plants, Plant Physiol., 104: 67-74. 26.El-Khallal, S. M. 2007. Induction and modulation of resistance in tomato plants against Fusarium wilt disease by bioagent fungi (arbuscular mycorrhiza) and/or hormonal elicitors (jasmine acid and salicylic acid): 1-changes in growth, some metabolic activities and endogenous hormones related to defense mechanism. Australian Journal of Basic and Applied Sciences, 1(4): 691-705. 27.Flor, H. H. 1971. Current status of the gene-for-gene concept. Ann. Rev. Phytopathol., 9: 275-296. 28.Frederick, K. R., Tung, J., Emerick, R. S., Masiarz, F. R., Chamberlain, S. H., Vasavada, A., Rosenberg, S., Chakraborty, S., Schopter, L. M. and Massey, V. 1990. Glucose oxidase from Aspergillus niger. Cloning, gene sequence, secretion from Saccharomyces cerevisiae and kinetic analysis of a yeast-derived enzyme. J Biol Chem., 265: 3793-3802. 29.Ganesan, V. and Thomas, G. 2001. Salicylic acid response in rice: influence of salicylic acid on H2O2 accumulation and oxidative stress. Plant Sci., 160: 1095-1106. 30.Grant, M. and Lamb, C. 2006. Systemic immunity. Plant Biology, 9(4): 414-420. 31.Greenberg, J. T. 1996. Programmed cell death: a way of life for plants. Proc. Natl. Acad. Sci., 93: 12094-12097. 32.Greenberg, J. T., Guo, A., Klessig, D. F. and Ausubel, F. M. 1994. Programmed cell death in plants: a pathogen-triggered response activated coordinately with multiple defense functions. Cell, 77: 551-563. 33.Hammond-Kosack, K. E. and Jones, J. D. G. 1997. Plant disease resistance genes. Annu. Rev. Plant Physiol. Plant Mol. Biol., 48: 575-607. 34.Harfouche, A. L., Rugini, E., Mencarelli, F., Botondi, R. and Muleo R. 2008. Salicylic acid induces H2O2 production and endochitinase gene expression but not ethylene biosynthesis in Castanea sativa in vitro model system. J Plant Physiol., 165: 734-744. 35.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. 36.Jabs, T., Dietrich, R. A. and Dangl, J. L. 1996. Initiation of runaway cell death in an Arabidopsis mutant by extracellular superoxide. Science, 273: 1853-1856. 37.Kachroo, A., He, Z., Patkar, R., Zhu, Q., Zhong, J., Li, D., Ronald, P., Lamb, C. and Chattoo, B. B. 2003. Induction of H2O2 in transgenic rice leads to cell death and enhanced resistance to both bacterial and fungal pathogens. Transgenic Res., 12: 577-586. 38.Kauss, H. 1987. Some aspects of calcium-dependent regulation in plant metabolism. Annu. Rev. Plant Physiol., 38: 47-72. 39.Kauss, H. and Jeblick, W. 1995. Pretreatment of parsely suspension cultures with salicylic acid enhances spontaneous and elicited production of H2O2. Plant Physiol., 108: 1171-1178. 40.Kazan, K., Murray, F., Goulter, K., Llewellyn, D. and Manners, J. 1998. Induction of cell death in transgenic plants expressing a fungal glucose oxidase. Mol Plant-Microbe Interact, 11: 555-562. 41.Keen, N. T. 1990. Gene-for-gene complementarity in plant-pathogen interactions. Annu. Rev. Genet, 24: 447-463. 42.Keen, N. T. 1992. The molecular biology of disease resistance. Plant MOI. Biol., 19: 109-122. 43.Kim, K. K., Flavel, D. R. and Papavizas, G. C. 1988. Identification of a metabolite produced by Talaromyces flavus as glucose oxidase and its role in the biocontrol of Verticillium dahliae. Phytopathology, 78: 488-492. 44.Lamb, C. and Dixon, R. A. 1997. The Oxidative burst in plant disease resistance. Ann. Rev. Plant Physiol. Plant Mol. Biol., 48: 251-275. 45.Lee, Y. H., Yoon, I. S., Suh, S. C. and Ki, H. I. 2002. Enhanced disease resistance in transgenic cabbage and tobacco expressing a glucose oxidase from Aspergillus niger. Plant Cell Rep., 20, 857-863. 46.Maruthasalam, S., Liu, Y. L., Sun, C. M., Chen, P. Y., Yu, C. W. and Lin, C. H. 2010. Constitutive expression of a fungal glucose oxidase gene in transgenic tobacco confers chilling tolerance through the activation of antioxidative defence system. Plant Cell Rep., 299: 14. 47.Mehdy, M. C. 1994. Active oxygen species in plant defense against pathogens. Plant Physiol., 105: 467-472. 48.Mittler, R., Del Pozo, O., Meisel, L. and Lam, E. 1997. Pathogeninduced programmed cell death in plants, a possible defense mechanism. Dev. Genet, 21: 279-289. 49.Montesano, M., Brader, G. and Palva, E. T. 2003. Pathogen derived elicitors: searching for receptors in plants. Molecular Plant Pathology, 4: 73-79. 50.Murashige, T. and Skoog, F. A. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant, 15: 473-497. 51.Murray, F., Llewellyn, D., Mcfadden, H., Last, D., Dennis, E. S. and Peacock, W. J. 1999. Expression of the Talaromyces flavus glucose oxidase gene in cotton and tobacco reduces fungal infection, but is also phytotoxic. Mol Breed, 5: 219-232. 52.O ' Kane, Gill, D., Boyd, V. P. and Burdon, R. 1996. Chilling, oxidative stress and antioxidant responses in Arabidopsis thaliana callus. Planta, 198: 371-377. 53.Olson, P. D. and Varner, J.E. 1993. Hydrogen peroxide and lignification. Plant J., 4: 887-892. 54.Peng, M. and Kuc, J. 1992. Peroxidase-generated hydrogen

peroxide as a source of antifungal activity in vitro and on tobacco leaf disks. Phytopathology, 82: 696-699. 55.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. 56.Rao, M. V., Paliyath, G., Ormrod, D. P., Murr, D. P. and Watkins, C. B. 1997. Influence of salicylic acid on H2O2 production, oxidative stress, and H2O2-metabolizing enzymes. Salicylic acid-Plant Soil mediated oxidative damage requires H2O2. Plant Physiol., 115: 137-149. 57.Ryals, J. A., Neuenschwander, U. H., Willits, M. G., Molina, A., Steiner, H. Y. and Hunt, M. D. 1996. Systemic acquired resistance. Plant Cell, 8: 1809-1819. 58.Sandermann, H., Ernst, D., Heller, W. and Langebartels, C. 1998. Ozone: An abiotic elicitor of plant defence reactions. Trends Plant Sci., 3: 47-50. 59.Shunja, T. K., Hanai, I., Galis, K., Suzuki, K., Matsuoka, H. and M. Soite 2007.Characterization of Nt chit IV, a class IV chitinase induced by β-1, 3-, 1-6-glucan elicitor from Alternaria alternate 102: Antagonistic effect of salicylic acid methyl jasmonate on the induction of Nt chit IV. Biochemical Biophysical Research Communication, 353: 311-317. 60.Staskawicz, B. J., Ausubel, F. M., Baker, B. J., Ellis, J. G. and Jones, J. D. 1995. Molecular genetics of plant disease resistance. Science, 268, 5211: 661-7. 61.Suvarnalatha, G., Rajendran, L., Ravishankar, G. A. and Venkataraman, L. V. 1994. Elicitation of anthocyanin production in cell cultures of carrot (Daucus carota L.) by using elicitors and abiotic stress. Biotechnol. Lett., 16: 1275-1280. 62.Uknes, S., Dincher, S., Friedrich, L., Negrotto, D., Williams, S., Thompson-Taylor, H., Potter, S., Ward, E. and Ryals, J. 1993. Regulation of pathogen-related protein-1a gene expression in tobacco. Plant Cell, 5: 159-169. 63.Ward, E. R., Uknes, S. J., Williams, S. C., Dincher, S. S., Wiederhold, D. L., Alexander, D. C., Ahl-Goy, P., Metraux, J. P. and Ryals, J. 1991. Coordinate gene activity in response to agents that induce systemic acquired resistance. Plant Cell, 3: 1085-1094. 64.Wu, G., Shortt, B. J., Lawrence, E. B., Len, J., Fitzsimmons, K. C., Levine, E. B., Raskin I et al. 1997. Activation of host defense mechanisms by elevated production of H2O2 in transgenic plants. Plant Physiol., 115: 427-435. 65.Wu, G., Shortt, B. J., Lawrence, E. B., Levine, E. B., Fitzsimmons, K. C. and Shah, D. M. 1995. Disease resistance conferred by expression of a gene encoding H2O2-generating glucose oxidase in transgenic potato plants. Plant Cell, 7: 1357-1368. 66.Wu, Z., Chen, L. J. and Long, Y. J. 2009. Analysis of ultrastructure and reactive oxygen species of hyperhydric garlic (Allium sativum L) shoots. In Vitro Cell Dev Biol-Plant, 45: 483-490. 67.Van Loon, L. C., Van Strien, E. A. 1999. The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. Physiol. Mol. Plant Pathol., 55: 85-97. 68.Zhao, J., Daves, L. C. and Verpoorte, R. 2005. Elicitor signal transduction leading to production of plant secondary metabolites. Biotechnol. Adv., 23: 283-333.