Expression and Characterization of Human Superoxide Dismutase Gene in Tomato Plants

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ABSTRACT

The extracellular superoxide dismutase (EC-SOD) is predominantly located in extracellular fluids and extracellular matrix of tissues and plays a central role to scavenge O2.- generated in extracellular space of mammals. The objective of this study is to transfer a human ec-sod gene into tomato plants to see if the SOD protein and activity are properly expressed, and then to investigate the responses of the transgenic plants under environmental stress. The human ec-sod gene was constructed and transformed into tomato plants (Lycopersicon esculentum L. cv. Known-You 301). Three different constructs were obtained from PCR amplification and cloning process. The first construct contained a signal peptide at the N-terminus of the sod gene for extracellular secretion. The second construct with no N-terminal signal peptide but with a sequence of KDEL located at the C-terminus of the sod gene for targeting to endoplasmic reticulum (ER). The last construct contained sod gene but with none of the signal peptide. These three constructs were introduced into tomato plants either by Agrobacterium-mediated transformation or by particle gun bombardment. The regeneration of non-transgenic Known-You 301 had the best regeneration rate in the MS medium containing 2 mgL-1 Benzylamino purine (BA) and 0.02 mgL-1 naphthaleneacetic acid (NAA). From the kanamycin sensitivity test on non-transgenic Known-You 301 tomato plants, it was found that 40 mgL-1 of kanamycin was high enough to stop the regeneration of tomato. The transformation results showed that 52 putative EC-SOD transgenic lines were regenerated from 2945 cotyledons, and the transformation rate is 1.5-1.8 %. Forty of them were positive by PCR amplification using the specific primers to nptII gene and to sod gene. The expression of EC-SOD in the putative transgenic lines were confirmed by RT-PCR amplification coupled with SmaI restriction digestion. Western blotting analysis was used to further detect the expression of the sod gene by using the anti-SOD antiserum specific against to the peptide from amino acid residue 221 to 235 of EC-SOD. Two clear protein bands with the molecular weight about 31 and 33 kDa were found in all the transgenic tomato lines. In the NBT activity assays, one extra band was observed in the transgenic plants. This pattern was disappearing after treated the gel with 3 mM KCN. The result suggested that the extra band may be the EC-SOD. Transgenic line 8-2 that contained the N terminal extracellular signal peptide of SOD was further check for the expression of SOD at 37°C. A specific protein about 31 kDa was observed at the second day of the heat treatment and was lasting to the fourth day. The protein band was still present in the transgenic plants three days after the plant moved back to the normal environment.

Keywords : Human Superoxide Dismutase ; tomato ; after

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REFERENCES

References 1. 王添成. 1997. 茄科蔬菜之抗病育種. 健康清潔植物培育研習會專刊. 中華民國植物病理學會出版頁 30-36. 2. 陳正次. 1994. 亞蔬中心耐熱抗病番茄育種之經歷（上）. 農藥世界 133:17-21. 3. 賴森雄、楊又迪. 1970. 番茄-台灣農家要覽（上）. 豐年社 p.1003-1013. 4. Agharbaoui, Z., Greer, A. F., and Tabaeizadeh, Z. (1995). Transformation of the wild tomato Lycopersicon chilense Dun. by Agrobacterium tumefaciens. Plant Cell Rep 15: 102-105. 5. Allen, R. D. (1995). Dissection of Oxidative Stress Tolerance Using Transgenic Plants. Plant Physiol 107: 1049-1054. 6. Allen, R. D., Webb, R. P., Schake, S. A. (1997). Use of transgenic plants to study antioxidant defenses. Free Radic Biol 23: 473-479. 7. Ames, B. N., and Saul, R. L. (1986). Oxidative DNA damage as related to cancer and aging. Prog Clin Biol Res. 209: 11-26. 8. Bartoli, C. G., Gomez, F., Martinez, D. E., and Guiamet, J. J. (2004). Mitochondria are the main target for oxidative damage in leaves of wheat (Triticum aestivum L.). J Exp Bot 55: 1663-1669. 9. Ben rejeb, I., Lenne, C., Leblance, N., Juliwn, J. L., Ammar, S. Bouzid, S., and Ayadi, A. (2004). Iron-superoxide dismutase and monodehydroascorbate reductase transcripts accumulate in response to internode rubbing in tomato. C. R. Biol. 327: 679-686. 10. Benedetto, M. T., Anzai, Y., and Gordon, J. W. (1991). Isolation and analysis of the mouse genomic sequence encoding Cu-Zn superoxide dismutase. Gene 99: 191-195. 11. Beyer, W. F. J., and Fridovich, I. (1991). In vivo competition between iron and manganese for occupancy of the active site region of the manganese-superoxide dismutase of Escherichia coli. J Biol Chem 266: 303-308. 12. Bonder, C. S., Knight, D., Hernandez-Saavedra, D., McCord, J. M., and Kubes, P. (2004). Chimeric SOD2/3 inhibits at the endothelial-neutrophil interface to limit vascular dysfunction in ischemia-reperfusion. Am J Physiol Gastrointest Liver Physiol. 287: 676-684. 13. Bowler, C., Slooten, L., Vandenbranden, S., De Rycke, R., Botterman, J., Sybesma, C., Van Montagu, M., and Inze, D. (1991). Manganese superoxide dismutase can reduce cellular damage mediated by oxygen radicals in transgenic plants. EMBO 10: 1723-1732. 14. Bowler, C., Van Camp, W., Van Montagu, M., and Inze, D. (1994). Superoxide dismutase in plants. Crit Rev Plant Sci 13: 199-218. 15. Bowler, R. P., and Crapo, J.D. (2002a). Oxidative stress in airways: is there a role for extracellular superoxide dismutase? Am J Respir Crit Care Med 166: S38-S43. 16. Bowler, R. P., Nicks, M., Olsen, D. A., Thogersen, I. B., Valinickova, Z., Hojrup, P., Franzusoff, A., Enghild, J, J., and Crapo, J. D. (2002b). Furin proteolytically processes the heparin-binding region of extracellular superoxide dismutase. J Biol Chem 227: 16505-16511. 17. 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. 18. Bryan, D. M., Stephen, R. B., Erni, H., and Olivier, L. (1996). Water-Deficit Tolerance and Field Performance of Transgenic Alfalfa Overexpressing Superoxide Dismutase. Plant Physiol 111: 1177-1181. 19. Butterfield, D. A., Hensley, K., Harris, M., Mattson, M., and Carney, J. (1994). beta-Amyloid peptide free radical fragments initiate synaptosomal lipoperoxidation in a sequence-specific fashion: implications to Alzheimer's disease. Biochem Biophys Res Commun 200: 710-715. 20. Cardinale, A, Filesi, I., Vetrugno, V., Pocchiari, M. S., and MS, B. S. (2005). Trapping prion protein in the endoplasmic reticulum impairs PrPC maturation and prevents PrPSc accumulation. J Biol Chem 280: 685-694. 21. Carlsson, L. M., Jonsson, J., Edlund, T., and Marklund, S.L. (1995). Mice lacking extracellular superoxide dismutase are more sensitive to hyperoxia. Proc Natl Acad Sci USA 92: 6264-6268. 22. Chen, E. P., Bittner, H. B., Davis, R. D., Folz, R. J., and Van Trigt, P. (1998). Extracellular superoxide dismutase transgene overexpression preserves postischemic myocardial function in isolated murine hearts. Circulation 94: 412-417. 23. Chyi, Y. S., and Phillips, G. C. (1987). High efficiency Agrobacteri-mediated transformation of Lycopersicon based on conditions favorable for regeneration. Plant Cell Rep 6: 105-108. 24. Chu, Y. S. I., Donald, D. L., Robert, M. W., Gerald, F. D., Yoshimasa, W., Frank, M. F., and Donald, D. H. (2003). Gene Transfer of Extracellular Superoxide Dismutase Reduces Arterial Pressure in Spontaneously Hypertensive Rats. Circulation Research. 92: 461. 25. Dahai, Z., and John, C. S. (1994). Differential accumulation of manganese-superoxide dismutase transcripts in maize in response to abscisic acid and high osmoticum. Plant Physiol 106: 173-178. 26. Davuluri, G. R., Tuinen, A., Mustilli, A. C., Manfredonia, A., Newman, R., Burgess, D., Brummell, D. A., King, S. R., Palys, J., Uhlig, J., Pennings, H. M., and Bowler, C. (2004). Manipulation of DET1 expression in tomato results in photomorphogenic phenotypes caused by post-transcriptional gene silencing. Plant J 40: 344-354. 27. Davuluri, G. R., van Tuinen, A., Fraser, P. D., Manfredonia, A., Newman, R., Burgess, D., Brummell, D. A., King, S. R., Palys, J., Uhlig, J., Bramley, P. M., Pennings, H. M., and Bowler, C. (2005). Fruit-specific RNAi-mediated suppression of DET1 enhances carotenoid and flavonoid content in tomatoes. Nat Biotechnol 23: 890-895. 28. Delannay, X., LaValle, B. J., Proksch, R. K., Fuchs, R. L., Sims, S. R., Greenplate, J. T., Marrone, P. G., Dodson, R. B., Augustine, J. J., Layton, J. G., and Fischhoff, D. A. (1989). Field performance of transgenic tomato plants expressing the Bacillus thuringiensis var. kurstaki insect control protein. Bio Technology 7: 1265-1269. 29. Delanty, N., and Dichter, M. A. (1998). Oxidative injury in the nervous system. Acta Neurol Scand. 98: 145-153. 30. De Cosa, B., Moar, W., Lee, S. B., Miller, M., Daniell, H. (2001). Overex pression of the Bt Cry2Aa2 operon in chloroplasts leads to formation of insecticidal crystals. Nat Biotechnol 19: 71-74. 31. Del Rio, L. A., Sandalio, L. M., Palma, J. M., Bueno, P., and Corpas, F. J. (1992). Metabolism of oxygen radicals in peroxisomes and cellular implications. Free Radic. Biol. Med. 13: 557-580. 32. Dolores, A., Marta, R., Mercedes, M., and Bartolome, S. (2001). Arabidopsis thaliana ecotype Cvi shows an increased tolerance to photo-oxidative stress and contains a new chloroplastic copper/zinc superoxide dismutase isoenzyme. J Exp Bot 52: 1417-1425. 33. DiSilvestro, R. A., Yang, F. L., and David, E. A. (1992). Species-specific heterogeneity for molecular weight estimates of serum extracellular superoxide dismutase activities. Comp Biochem Physiol B 101: 531-534. 34. Edlund, A. T., Edlund, K., Hjalmarsson, S. L., Marklund, J., Sandstrom, M., and Stromqvist, L. T. (1992). A nonglycosylated extracellular superoxide dismutase variant. Biochem J 288: 451-456. 35. Fahn, S., and Cohen, G. (1992). The oxidant stress hypothesis in Parkinson's disease: evidence supporting it. Ann Neurol 32: 804-812. 36. Fattman, C. L., Enghild, J. J., Crapo, J. D., Schaefer, L. M., Valnickova, Z., and Oury, T. D. (2000). Purification and characterization of extracellular superoxide dismutase in mouse lung. Biochem. Biophys. Res. Commun. 275: 542-548. 37. Fennell, J. P., Brosnan, M. J., Frater, A. J., Hamilton, C. A., Alexander, M. Y., Nicklin, S. A., Heistad, D. D., Baker, A. H., and Dominiczak, A. F. (2002). Adenovirus-mediated

overexpression of extracellular superoxide dismutase improves endothelial dysfunction in a rat model of hypertension. Gene Ther 9: 110-117. 38. Fillatti, J. J., Kiser, J., Rose, R., and Comai, L. (1987). Efficient transfer of a glyphosate tolerance gene into tomato using a binary Agrobacterium tumefaciens vector. Bio Technology 5: 726-730. 39. Fischhoff, D. A., Bowdish, K. S., Perlak, F. J., Marrone, P. G., McCormick, S. M., Niedermayer, J. G., Dean, D. A., Kusano-Kretzmer, K., Mayer, E. J., Rochester, D. E., Rogers, S. G., and Fraley, R. T. (1987). Insect tolerant transgenic tomato plants. Bio Technology 5: 807-813. 40. Flohe, L., and Otting, F. (1984). Methods in Enzymology: Oxygen Radicals in Biological Systems (Packer, L., ed) , Academic Press, New York 93-104. 41. Forman, H. J., and Fridovich, I. (1973). On the stability of bovine superoxide dismutase. The effects of metals. J Biol Chem 248: 2645-2649. 42. Frary, A., and Earle, E. D. (1996). An examination of factors affecting the efficiency of Agrobacterium-mediated transformation of tomato. Plant Cell Rep 16: 235-240. 43. Frary, A., and Van Eck, J. (2005). Organogenesis from transformed tomato explants. Methods Mol Biol 286: 141-150. 44. Fukai, T., Siegfried, M. U., Cheng, G. K., and Harrison. D. G. (2000). Regulation of the vascular extracrllular superoxide dismutase by nitric oxide and exericise training. J Clin Invest 105: 1631-1639. 45. Fukai, T., Folz, R. J., Landmesser, U., and Harrison, D. G. (2002). Extracellular superoxide dismutase and cardiovascular disease. Cardiovasc Res 55: 239-249. 46. Fridovich, I. (1995). Superoxide radical and superoxide dismutases. Annu Rev Biochem. 64: 97-112. 47. Gao, Y., Ma, Y., Li, M., Cheng, T., Li, S.W., Zhang, J., and Xia, N. S. (2003). Oral immunization of animals with transgenic cherry tomatillo expressing HBsAg. World J Gastroenterol 9: 996-1002. 48. Giddings, G. (2001). Transgenic plants as protein factories. Curr Opin Biotechnol 12: 450-454. 49. Gil, F., Brun, A., Wigdorovitz, A., Catala, R., Martinez-Torrecuadrada, J. L., Casal, I., Salinas, J., Borca, M.V., and Escribano, J. M. (2001). High-yield expression of a viral peptide vaccine in transgenic plants. FEBS 488: 13-17. 50. Gupta, A. S., Webb, R. P., Holaday, A. S., Allen, R. D. (1993). Overexpression of Superoxide Dismutase Protects Plants from Oxidative Stress (Induction of Ascorbate Peroxidase in Superoxide Dismutase-Overexpressing Plants). Plant Physiol 103: 1067-1073. 51. Halliwell, B. and Gutteridge, J. M. (1990). Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol 186: 1-85. 52. Halliwell, B., Gutteridge, J. M., and Cross, C. E. (1992). Free radicals, antioxidants, and human disease: where are we now? J Lab Clin Med 119: 598-620. 53. Halliwell, B. (1989). Free radicals, reactive oxygen species and human disease: a critical evalution with special reference to atherosclerosis. Br. J Exp Pathol 70: 737-757. 54. Halliwell, B. (1993). Free radicals and vascular disease: how much do we know? BMJ 307: 885-886. 55. Hanin, M., Volrath, S., Bogucki, A., Briker, M., Ward, E., and Paszkowski, J. (2001). Gene targeting in Arabidopsis. Plant J 28: 671-677. 56. Harish, K., and Vinod, K. (2004). Tomato expressing Cry1A(b) insecticidal protein from Bacillus thuringiensis protected against tomato fruit borer, Helicoverpa armigera (H.ubner) (Lepidoptera: Noctuidae) damage in the laboratory, greenhouse and field. Crop Protection 23: 135-139. 57. Herouart, D., Bowler, C., Willekens, H., Van Camp, W., Slooten, L., Van Montagu, M., and Inze, D. (1993). Genetic engineeringof oxidative stress resistance in higher plants. Philos Trans R Soc Lond-Biol Sci 342: 235-240. 58. Hialmarsson, K. Marklund, S. L. Engstrom, A., and Edlund, T. (1987). Isolation and sequence of complementary DNA encoding human extracellular superoxide dismutase. Proc. Natl. Acad. Sci. USA 84: 6340 – 6344. 59. Hjalmarsson, K., Marklund, S. L., Engstrom, A., and Edlund, T. (1987). Isolation and sequence of complementary DNA encoding human extracellular superoxide dismutase. Proc Natl Acad Sci USA 84: 6340-6344. 60. Hood, E. E., Woodard, S. L., and Horn, M. E. (2002). Monoclonal antibody manufacturing in transgenic plants--myths and realities. Curr Opin Biotechnol. 13: 630-635. 61. Hsu, J. L., Visner, G. A., Burr, I. A., and Nick, H. S. (1992). Rat copper/zinc superoxide dismutase gene: isolation, characterization, and species comparison. Biochem. Biophys Res Commun. 186: 936-943. 62. Hopkin, K. A., Papazian, M. A., and Steinman, H. M. (1992). Functional differences between manganese and iron superoxide dismutases in Escherichia coli K-12. J Biol Chem. 267: 24253-24258. 63. Hu, W., and Phillips, G. C. (2001). Combination of overgrowyh-control and antibiotics improves agrobacterium tumefaciens-mediated transformation efficiency for cultivated tomato (L. esculentum). In vitro cell Biol Plant 37: 12-18. 64. Igor, N. Z., Thomas, J. M., and Rodney, J. F. (2002). Superoxide dismutase multigene family: a compararison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. Free Redical Biology and Medicine. 33: 337-349. 65. Inako, T., Matsumura, Y., and Tsucido, T. (1998). Molecular cloning and nucleotide sequence of the superoxide dismutase gene and characterization of its product from Bacillus subtilis. J. Bacteriol. 180: 3697-3703. 66. Jannick Dyrlov, B., Henrik, N., Gunnar von, H. and Soren, B. (2004). Improved prediction of signal peptides: SignalP 3.0. J. Mol. Biol. 340: 783-795. 67. Kanematsu, S., and Asada, K. (1991). Chloroplast and cytosol isozymes of CuZn-superoxide dismutase: their characteristic amino acid sequences. Free Radic Res Commun. 1: 383-390. 68. Kendall, E. J., and McKersie, B. D. (1989). Free radical and freezing injury to cell membranes of winter wheat. Plant Physiol 76: 86-94. 69. Khanna-Chopra, R., and Sabarinath, S. (2004). Heat-stable chloroplastic Cu/Zn superoxide dismutase in Chenopodium murale. Biochem Biophys Res Commun. 320: 1187-1192. 70. Kim, Y. H., Yoo, H. Y., Jung, G., Kim, J. T., and Rho, H. M. (1993). Isolation and analysis of the rat genomic sequence encoding Cu/Zn superoxide dismutase. Gene 133: 267-271. 71. Ku, H. H., Brunk, U. T., and Sohal, R. S. (1993). Relationship between mitochondrial superoxide and hydrogen peroxide production and longevity of mammalian species. Free Radic Biol Med 15: 621-627. 72. Landmesser, U., Merten, R., Spiekermann, S., Buttner, K., Drexler, H., and Hornig, B. (2000). Vascular extracellular superoxide dismutase activity in patients with coronary artery disease: relation to endothelium-dependent vasodilation. Circulation. 101: 2264-2270. 73. Laursen, J. B., Rajagopalan, S., Galis, Z., Tarpey, M., Freeman, B. A., and Harrison, D.G. (1997). Role of superoxide in angiotensin II-induced but not catecholamine-induced hypertension. Circulation. 95: 588-593. 74. Lauterslager, T. G., Florack, D. E., van der Wal, T. J., Molthoff, J. W., Langeveld, J. P., Bosch, D., Boersma, W. J., and Hilgers, L. A. (2001). Oral immunization of naive and primed animals with transgenic potato tubers expressing LT-B. Vaccine 19: 2749-2755. 75. Lebovitz, R. M., Zhang, H., Vogel, H., Cartwright, J. J., Dionne, L., Lu, N., Huang, S., and Matzuk, M. M. (1996). Neurodegeneration, myocardial injury, and perinatal death in mitochondrial superoxide dismutase-deficient mice. Proc. Natl. Acad. Sci. USA 93: 9782-9787. 76. Lee,

K. W., Meyer, N., and Ortwerth, B. J. (1999). Chromatographic comparison of the UVA sensitizers present in brunescent cataracts and in calf lens proteins ascorbylated in vitro. Exp Eye Res. 69: 375-384. 77. Levanon, D., Lieman-Hurwitz, J., Dafni, N., Wigderson, M., Sherman, L., Bernstein, Y., Laver-Rudich, Z., Danciger, E., Stein, O., and Groner, Y. (1985). Architecture and anatomy of the chromosomal locus in human chromosome 21 encoding the Cu/Zn superoxide dismutase. EMBO J. 4: 77-84. 78. Levine, A., Tenhaken, R., Dixon, R., and Lamb, C. (1994). H2O2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell. 79: 583-593. 79. Libik, M., Konieczny, R., Pater, B., Slesak, I., and Miszalski,Z. (2004). Differences in the activities of some antioxidant enzymes and in H2O2 content during rhizogenesis and somatic embryogenesis in callus cultures of the ice plant. Plant Cell Rep. 23: 834-841. 80. Lim, J. H., Yu, Y. G., Choi, I. G., Ryu, J. R., Ahn, B. Y., Kim, S. H., and Han. Y. S. (1997). Cloning and expression of superoxide dismutase from Aqufiex pyrophilus, a hyperthermophilic bacterium. FEBS Lett 406: 142-146. 81. Ling, H. Q., Krise. leit, D., and Ganal, M. G. (1998). Effect of ticarcillin/potassium clavulanate on callus growth and shoot regeneration in Agrobacterium-mediated transformation of tomato (Lycopersicon esculentum Mill). Plant Cell Rep 17: 843-847. 82. Maria, C. S., Revilla, E., Ayala, A., de la Cruz, C. P., and Machado, A. (1995). Changes in the histidine residues of Cu/Zn superoxide dismutase during aging. FEBS Lett. 374: 85-88. 83. Marklund, S. L. (1982). Human copper-containing superoxide dismutase of high molecular weight. Proc Natl Acad Sci USA 79: 7634-7638. 84. Marklund, S. L., Holme, E., and Hellner, L. (1982). Superoxide dismutase in extracellular fluids. Clin Chim Acta 126: 41-51. 85. Marklund, S. L. (1984a). Extracellular suprtoxidase dismutase and other superoxide dismutase isoenzymes in tissue from nine mammalian species. Biochem J 222: 649-655. 86. Marklund, S. L. (1984b). Extracellular superoxide dismutase in human tissues and human cell lines. J Clin Invest 74: 1398-1403. 87. Marklund, S. L., Bjelle, A., and Elmqvist, L. G. (1986). Superoxide dismutase isoenzymes of the synovial fluid in rheumatoid arthritis and in reactive arthritides. Ann Rheum Dis 45: 847-851. 88. Mason, H. S., Lam, D. M., and Arntzen, C. J. (1992). Expression of hepatitis B surface antigen in transgenic plants. Proc Natl Acad Sci USA 89: 11745-11749. 89. McCord, J. M., and Fridovich, I. (1969). The utility of superoxide dismutase in studying free radical reactions. I. Radicals generated by the interaction of sulfite, dimethyl sulfoxide, and oxygen. J Biol Chem 244: 6056-6063. 90. McCord, J. M., and Fridovich, I. (1970). The utility of superoxide dismutase in studying free radical reactions. II. The mechanism of the mediation of cytochromec reduction by a variety of electron carriers. J Biol Chem 245: 1374-1377. 91. McCormick, S., Niedermeyer, J., Fry, J., Barnason, A., Horsch, R., and Fraley, R. (1986). Leaf disc transformation of cultivated tomato (L.esculentum) using Agrobacterium tumefaciens. Plant Cell Rep 5: 81-84. 92. McGaughey, W. H., and Whalon, M. E. (1992). Managing insect resistance to Bacillus thuringiensis toxins. Science 258: 1451-1455. 93. McKersie, B. D., Hoekstra, F. A., and Krieg, L. C. (1990). Differences in the susceptibility of plant membrane lipids to peroxidation. Biochim Biophys Acta 1030: 119-126. 94. McKersie, B. 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. 95. McKersie, B. D., Chen, Y., de Beus, M., Bowley, S. R., Bowler, C., Inze, D., D'Halluin, K., and Botterman, J. (1993). Superoxide dismutase enhances tolerance of freezing stress in transgenic alfalfa (Medicago sativa L.). Plant Physiol 103: 1155-1163. 96. Mehdy, M. C. (1994). Active oxygen species in plant defense against pathogens. Plant Physiol 105: 467-472. 97. Miller, A. F. (2004). Superoxide dismutases: active sites that save, but a protein that kills. Curr Opin Chem Biol. 8: 162-168. 98. Monk, L. S., Fagerstedt, K.V., and Crawford, R. M. M. (1989). Oxygen toxicity and superoxide dismutase as an antioxidant in physiological stress. Plant Physiol 76: 456-459. 99. Murashige, T. and F. Skoog. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15: 473-497. 100. Neill, S., Desikan, R., and Hancock, J. (2002). Hydrogen peroxide signalling. Curr Opin Plant Biol. 5: 388-395. 101. Netto, C. B., Siqueira, I. R., Fochesatto, C., Portela, L. V., da Purificacao Tavares, M., Souza, D. O., Giugliani, R., and Goncalves, C. A. (2004). S100B content and SOD activity in amniotic fluid of pregnancies with Down syndrome. Clin Biochem. 37: 134-137. 102. Ogawa, K., Kanematsu, S., and Asada, K. (1996). Intra- and extra-cellular localization of " cytosolic " superoxide dismutase in spinach leaf and hypocotyl. Plant Cell Physiol. 37: 790-799. 103. Ohara, Y., Peterson, T. E., and Harrison D. G., (1993). Hypercholesterolemia increase endothelial superoxide anion production. J.Clin. Inverts. 91: 2546-2551. 104. Olof, E., Henrik, N., and Gunnar von, H. (1999). ChloroP, a neural network-based method for predicting chloroplast transit peptides and their cleavage sites. Protein Science. 8: 978-984. 105. Olsen, D. A., Petersen, S. V., Oury, T. D., Valnickova, Z., Thogersen, I. B., Kristensen, T., Bowler, R. P., Crapo, J. D., and Enghild, J. J. (2004). The intracellular proteolytic processing of extracellular superoxide dismutase (EC-SOD) is a two-step event. J Biol Chem. 279: 22152-22157. 106. Ookawara, T., Kizaki, T., Ohishi, S., Yamamoto, M., Matsubara, O., and Ohno, H. (1997). Purification and subunit structure of extracellular superoxide dismutase from mouse lung tissue. Arch Biochem Biophys. 340: 299-304. 107. Orr, W. C., and Sohal, R. S. (1994). Extension of life-span by overexpression of superoxide dismutase and catalase in Drosophila melanogaster. Science 263: 1128-1130. 108. Oury, T. D., Day, B. J., and Crapo, J. D. (1996a). Extracellular superoxide dismutase in vessels and airways of humans and baboons. Free Radic. Biol. Med. 20: 957-965. 109. Oury, T. D., Day, B. J., and Crapo, J. D. (1996b). Extracellular superoxide dismutase in vessels and airways of humans and baboons. Free Radic Biol Med. 20: 957-65. 110. Pitcher, L. H., Zilinskas, B. A. (1996). Overexpression of Copper/Zinc Superoxide Dismutase in the Cytosol of Transgenic Tobacco Confers Partial Resistance to Ozone-Induced Foliar Necrosis. Plant Physiol. 110: 583-588. 111. Pinhero, R. G., Rao, M. V., Paliyath, G., Murr, D. P., and Fletcher, R. A. (1997). Changes in Activities of Antioxidant Enzymes and Their Relationship to Genetic and Paclobutrazol-Induced Chilling Tolerance of Maize Seedlings. Plant Physiol 114: 695-704. 112. Rodney, J. F., and James, D. C. (1994). Extracellular superoxide dismutase (SOD3): Tissue-specific expression, genomic characterization, and computer-assisted sequence analysis of the human EC-SOD gene. Genomic. 22: 162-171. 113. Romanos, M. A., Scorer, C. A., and Clare, J. J. (1992). Foreign gene expression in yeast: a review. Yeast 8: 423-488. 114. Rosen, D. R., Siddique, T., Patterson, D., Figlewicz, D. A., Sapp, P., Hentati, A., Donaldson, D., Goto, J., O'Regan, J. P., and Deng, H. X. (1993). Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic

lateral sclerosis. Nature 362: 59-62. 115. Scandalios, J. G. (1993). Oxygen Stress and Superoxide Dismutases. Plant Physiol 101: 7-12. 116. Schinkel, H., Streller, S., and Wingsle, G. (1998). Multiple forms of extracellular superoxide dismutase in needles, stem tissues and seedlings of Scots pine. J Exp Bot 49: 931-936. 117. Senaratna, T., McKersie, B. D., and Stinson, R. H. (1985a). Simulation of dehydration injury to membranes from soybean axes by free radicals. Plant Physiol 77: 472-474. 118. Senaratna, T., McKersie, B. D., and Stinson, R. H. (1985b). Antioxidant levels in germinating soybean seed axes in relation to free radical and dehydration tolerance. Plant Physiol 78: 168-171. 119. Shirzadegan, M., Christie, P., and Seemann, J. R. (1991). An efficient method for isolation of RNA from tissue cultured plant cells. Nucleic Acids Res. 19: 6055. 120. Stromqvist, M., Holgersson, J., and Samuelsson. (1991). Glycosylation of extracellilar superoxide dismutase studied by high-performance liquid chromatography and mass spectrometry. J Chromatogr 548: 293-301. 121. Tainer, J. A., Getzoff, E. D., Richardson, J. S., and Richardson, D. C. (1983). Structure and mechanism of copper, zinc superoxide dismutase. Nature 306: 284-287. 122. Tanaka, Y., Hibino, T., Hayashi, Y., Tanaka, A., Kishitani, S., Takabe, T., Yokota, S., and Takabe, T. (1999). Salt tolerance of transgenic rice overexpressing yeast mitochondrial Mn-SOD in chloroplasts. Plant Sci 148: 131-138. 123. Terada, R., Urawa, H., Inagaki, Y., Tsugane, K., and Iida, S. (2002). Efficient gene targeting by homologous recombination in rice. Nat Biotechnol 20: 1030-1034. 124. Van Camp, W., Willekens, H., Bowler, C., van Montagu, M., and Inze, D. (1994). Elevated levels of superoxide dismutase protect transgenic plants against ozone damage. Biotechnology 12: 165-168. 125. Van Camp, W., Capiau, K., Van Montagu, M., Inze, D., and Slooten, L. (1996). Enhancement of oxidative stress tolerance in transgenic tobacco plants overproducing Fe-superoxide dismutase in chloroplasts. Plant Physiol. 112: 1703-1714. 126. Van Poppel, G., and van den Berg, H. (1997). Vitamins and cancer. Cancer Lett. 114: 195-202. 127. Van Roekel, J. S. C., Damm, B., Melchers, L. S., and Hoekema, A. (1993). Factors influencing transformation frequency of tomato (Lycopersicon esculentum). Plant Cell Rep 12: 644-647. 128. Vance, C. K., and Miller, A. F. (2001). Novel insights into the basis for Escherichia coli superoxide dismutase's metal ion specificity from Mn-substituted FeSOD and its very high E(m). Biochemistry 40: 13079-13087. 129. Verword, T. C., Dekker, B. M., and Hoekema, A. (1989). A small-scale procedure for the rapid isolation of plant RNAs. Nucl. Acids. Res. 17: 2362. 130. Vidya, C. S. S., Manoharan, M., Kumar, C. T. R., Savithri, H. S., and Sita, G. L. (2000). Agrobacterium-mediated transformation of tomato (Lycopersicon esculentum var. Pusa Ruby) with coat-protein gene of Physalis mottle tymovirus. J Plant Physiol 156: 106-110. 131. Walmsley, A. M., and Arntzen, C. J. (2000). Plants for delivery of edible vaccines. Curr Opin Biotechnol 11: 126-129. 132. Wang, Y., Ying, Y., Chena, J., and Wang, X. (2004). Transgenic Arabidopsis overexpressing Mn-SOD enhanced salt-tolerance. Plant Sci 167: 671 – 677. 133. Weisiger, R.A., Fridovich, I. (1973). Mitochondrial superoxide simutase. Site of synthesis and intramitochondrial localization. J Biol Chem. 248: 4793-4796. 134. Wheeler, M. D., Smutney, O. M., and Samulski, R. J. (2003). Secretion of extracellular superoxide dismutase from muscle transduced with recombinant adenovirus inhibits the growth of B16 melanomas in mice. Mol Cancer Res 1:871-881. 135. White, C. R., Brock, T. A., Chang, L. Y., Crapo, J., Briscoe, P. Ku, D. Bradley. W. A., Gianturco, S. H., Gore, J., Freeman, B. A., and Tarpey, M. M., (1994). Superoxide and peroxynitrite in atherosclerosis. Proc. Natl. Acad. Sci. U.S.A. 91: 1044-1048. 136. Williamson, J. D., and Scandalios, J. G. (1992). Differential response of maize catalases to abscisic acid: Vp1 transcriptional activator is not required for abscisic acid-regulated Cat1 expression. Proc Natl Acad Sci USA 89: 8842-8846. 137. Youn, H. D., Kim, E. J., Roe, J. H., Hah, Y. C., Kang, S. O. (1996). A novel nickel-containing superoxide dismutase from Streptomyces spp. Biochem J. 318: 889-896. 138. Zhu, D., and Scandalios, J. G. (1992). Expression of the maize MnSod (Sod3) gene in MnSOD-deficient yeast rescues the mutant yeast under oxidative stress. Genetics. 131: 803-809.