Expression of a Human Extracellular Superoxide Dismutase Gene by Turnip Mosaic Viral Vector

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ABSTRACT

Active oxygen species (AOS), such as superoxide(O2.-) are generated as by-products of normal metabolism in aerobic organisms. Superoxide dismutase (SOD) is the first line of defense to eliminate superoxide in the cellular environment. Extracellular SOD (EC-SOD), an isoform of SOD, is predominantly located in blood and extracellular matrix of tissue, and play a role to scavenge O2.- generated in extracellular space. Because SOD is a highly economic value protein, the objective of our study is to use Turnip mosaic virus (TuMV) viral vector to express human ec-sod gene in plant system. Four TuMV-ECSOD recombinant viruses were obtained by replacing the NSs gene in TuMV-NSs with ec-sod constructs. There were four different ec-sod constructs with or without a signal peptide at the N-terminus, and with or without an endoplasmic reticulum (ER) retention signal KDEL at the carboxyl-terminus. The resulting recombinant virus TuMV-ECSOD-S expressed the EC-SOD protein with a secreted N-terminal extracellular (EC) signal peptide. Recombinant TuMV-ECSOD-SK contained the EC-SOD with a secreted N-terminal signal peptide and a KDEL signal at C-terminus. Recombinant TuMV-ECSOD-N contained none of the signal peptide at both ends and recombinant TuMV-ECSOD-K had the EC-SOD protein with a KDEL at C-terminus. The four TuMV-ECSOD recombinant viruses were introduced into plants of Chenopodium quinoa Willd. All plants developed local lesion symptoms at 5 to 7 days post inoculation, except for the plants inoculated by TuMV-ECSOD-SK shown one day delay of symptom expression. The size of the local lesions on plants of quinoa infected by TuMV-ECSOD were similar to the plants inoculated by TuMV-NSs. Local lesions were collected and then inoculated into systemic hosts Brassica chinensis L. and Brassica chinensis L. CV. At approximately 10 to 21 days, the recombinants TuMV-ECSOD-N and TuMV-ECSOD-K inoculated plants showed mosaic, with some necrosis and hard, crisp systematic symptom and finally the entire plant dwarf, while the plants inoculated with the local lesions collected from TuMV-ECSOD-S and TuMV-ECSOD-Sk infected Chenopodium quinoa plants showed no symptom. Western blotting analyses with the antisera against EC-SOD and TuMV, respectively, were able to detect either the EC-SOD protein with the molecular weight of 24 kDa or TuMV coat protein with the molecular weight of 35 kDa. The results showed that TuMV-ECSOD-N and TuMV-ECSOD-K were infectious and were able to express EC-SOD protein correctly. In SOD activity assays with NBT, Riboflavin, KCN and H2O2 , the protein of CuZn-SOD extracted from plants inoculated with TuMV-ECSOD showed higher expression than those plants inoculated with TuMV-NSs or healthy plants. The results suggest that the expression of CuZn-SOD in plants can be stimulated by the inoculation of recombinant TuMV-ECSOD. A very faint extra SOD protein under the bands of CuZn-SOD was observed from the extracts of TuMV-ECSOD infected plant was considered as the EC-SOD protein. In addition, the TuMV-ECSOD infected plants showed better resistance to herbicide paraquat.

Keywords : Extracellular SOD (EC-SOD) ; Turnip mosaic virus (TuMV) ; Chenopodium quinoa Willd. ; Brassica chinensis L. ; Brassica chinensis L. CV. ; Paraquat

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