Plant Physiology and Biochemistry 109 (2016) 20–27

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Overexpression of *CuZnSOD* and *APX* enhance salt stress tolerance in sweet potato

Hui Yan [a], Qiang Li [a, *], Sung-Chul Park [b], Xin Wang [a], Ya-ju Liu [a], Yun-gang Zhang [a], Wei Tang [a], Meng Kou [a], Dai-fu Ma [a]

[a] Xuzhou Institute of Agricultural Sciences in Jiangsu Xuhuai District, Key Laboratory for Biology and Genetic Breeding of Sweetpotato (Xuzhou), Ministry of Agriculture/Jiangsu Xuzhou Sweetpotato Research Center, 221131, People's Republic of China
[b] Environmental Biotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Oun-dong 52, Yusong-gu, Daejeon, 305-806, Republic of Korea

ARTICLE INFO

Article history:
Received 20 May 2016
Received in revised form
2 September 2016
Accepted 2 September 2016
Available online 4 September 2016

Keywords:
Sweet potato
CuZnSOD
APX
Transgenic plants
Salt stress

ABSTRACT

Abiotic stresses cause accumulation of reactive oxygen species (ROS) in plants, CuZnSOD and APX are first line defenses against ROS caused by oxidative stress. In this study, *CuZnSOD* and *APX* were transferred into salt sensitive sweet potato (cv. Xushu 55–2) under control of stress inducible *SWPA2* promoter and tolerance to salt stress was evaluated. When 100 mM NaCl was used to treat stem cuttings, transgenic plants showed enhanced tolerance compared to wild type (WT) plants. Rooting was significantly retarded in WT plants whereas all transgenic plants had significantly enhanced root growth under salt stress. Integration of SOD gene was confirmed by southern blot analysis, and the copy number ranged from 1 to 3. The expression levels of *CuZnSOD* and *APX* in transgenic plants were significantly increased up to 13.3 and 7.8 folds to WT under salinity conditions, respectively. SOD and APX activity and ROS staining showed enzyme activities of transgenic plants were increased under salt stress. These results show that *CuZnSOD* and *APX* have important roles in enhancing the salt tolerance of sweet potato.

1. Introduction

Plant growth is influenced by many factors. Although plants have evolved adaptations to many abiotic challenges, these stresses often cause over 50% crop loss (Vinocur and Altman, 2005). Salinity is major limiting factors to crop productivity. Saline areas are widely distributed and approximately 20% of the available agriculture land is under salt stress (Neera and Shikha, 2011).

Salinity disturbs plant ionic equilibrium and can cause severe stress to normal osmotic regulation (Adams et al., 1992; Zhu, 2002; Zhao et al., 2003). The generation and scavenging of reactive oxygen species (ROS), such as superoxide radical anions ($O_2^{\bullet -}$), hydroxyl radicals (•OH), singlet oxygen ($^1O_2$), and hydrogen peroxide (H_2O_2) generated under salinity stress are usually in dynamic equilibrium in plants. ROS are part of a signaling pathway and cellular response but excessive ROS levels cause physiological and structural damage to cells (Asada, 1999; Ren et al., 2000; Takemura et al., 2002). ROS scavenging is therefore important for plant survival and growth under salinity stress conditions.

Transgenic plants that overexpressed certain antioxidative enzymes have enhanced stress resistance and the level of resistance is positively correlated with antioxidative enzyme concentration (Dhindsa et al., 1981; Hemandez et al., 2000; Mittova et al., 2003; Li et al., 2003). Superoxide dismutase (SOD), glutathione peroxidase, and catalase are key antioxidative enzymes countering the damaging effects of ROS. Superoxide dismutase converts the superoxide radical to H_2O_2, while ascorbate peroxidase (APX) catalyses the conversion of H_2O_2 to water and oxygen (Dhindsa et al., 1981). Kwon et al. demonstrated that simultaneous expression of *CuZnSOD* and *APX* in tobacco chloroplasts enhanced tolerance to methyl viologen (MV) stress compared to the tolerance provided by expression of the individual genes (Kwon et al., 2002). Transgenic potato and *Arabidopsis* exhibited similar phenomenon (Tang et al., 2006; Shafi et al., 2015). Simultaneous expression of *CuZnSOD* and *APX* in sweet potato resulted to enhanced protection of transgenic plants compared with non-transgenic plants under oxidative stress, chilling, and sulfur dioxide exposure (Lim et al., 2007; Kim et al., 2015). The enhanced salt tolerance of transgenic

* Corresponding author.
E-mail address: instrong@163.com (Q. Li).

http://dx.doi.org/10.1016/j.plaphy.2016.09.003
0981-9428/

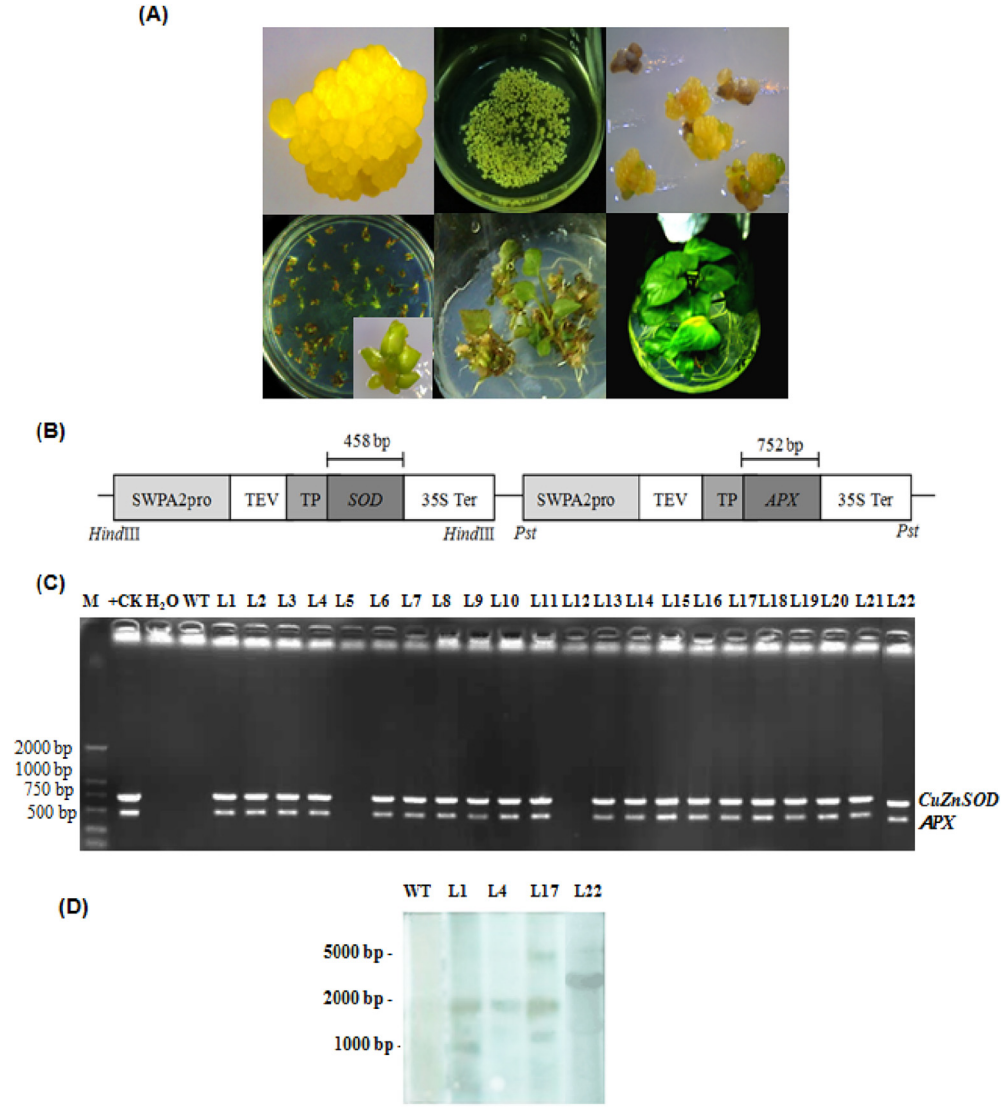

Fig. 1. Generation and gene analyses of transgenic sweet potato. A. Transformation, selection, and regeneration of sweetpotato cv. Xushu55-2. B. Plant transformation vector. *SWPA2pro*: sweetpotato peroxidase promoter, *SOD*: cassava Cu/Zn superoxide dismutase (AF170297.1), *APX*: pea ascorbate peroxidase (M93051.1), *TEV*: tobacco etch virus 5′-untranslated region, *35S Ter*: CaMV 35S terminator, *TP*: chloroplast-targeted transit peptide. C. PCR detection of regenerated sweetpotato lines. M: 2-kb DNA ladder; +CK: vector pCAMBIA2300 contains *CuZnSOD* and *APX* for positive control; WT: non-transgenic plant for negative control; L1-L4, L6-L11, L13-L22 positive sweetpotato plants expressing two target genes; L5, L12 negative plants lacking target gene expression. D. Southern blot analysis of *CuZnSOD*. DNA was digested with *HindIII* and hybridized with DIG-labeled *CuZnSOD* gene probe. WT: non-transgenic plant; L1, L4, L17, L22: transgenic lines.

sweet potato plants such as those derived from the salt sensitive cultivar Xushu 55-2 has not been determined.

A promoter is essential for driving the transcription of foreign genes in plants. Generally, CaMVA 35S promoter is most commonly used. The use of a stress-inducible promoter can provide a more precise regulation of expression. As a strong oxidative stress-inducible POD (*SWPA2*) from cultured cells of sweetpotato, its function have characterized in transgenic tobacco plants (Kim et al., 2003).

Sweet potato [*Ipomoea batatas* (L.) Lam.] is a nutritious and starch-rich food. It has also been used as a renewable raw material for the production of ethanol. Saline coastal lands are areas, in many countries, whose development and use can help improve food security. Crop varieties with greater tolerance to saline conditions are needed to use these saline areas for food production. In this study, Xushu 55−2, obtained from the Xuzhou Sweetpotato Research Center, China and was selected as a sweet potato cultivar which with high quality starch but significant salt sensitivity. We successfully generated transgenic sweet potato plants expressing 2 antioxidant enzyme genes and evaluated the salinity tolerance of the transgenic plants.

2. Materials and methods

2.1. Transformation and regeneration of sweet potato

Embryogenic calli were induced from Xushu 55−2 on Murashige and Skoog (MS) medium containing 30 g L^{-1} sucrose + 2.0 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) (abbreviated it as MSD medium). Establishment of embryogenic suspension cultures of Xushu 55−2 was done following the method described by Liu et al. (1997). Sixteen weeks after induction, cell aggregates were sub-cultured for 3 d in a suspension culture medium then subjected to transformation.

The *Agrobacterium tumefaciens* strain EHA105 harboring a binary vector pCAMBIA2300 (Fig. 1B) was used. The expression of