Transgenic Crops with Enhanced Tolerance to Multiple Environmental Stresses for Sustainable Agriculture

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Abstract

Oxidative stress derived from reactive oxygen species (ROS) is one of the major damaging factors in plants exposed to environmental stress. In order to develop various transgenic crops such as sweetpotato (*Ipomoea batatas* L. Lam), potato (*Solanum tuberosum* L.) and tall fescue (*Festuca arundinacea* Schreb.) plants with enhanced tolerance to multiple stresses, the genes of both Cu/Zn superoxide dismutase and ascorbate peroxidase were expressed in chloroplasts under the control of an oxidative stress-inducible peroxidase (*SWPA2*) promoter (referred to SSA plants). SSA plants showed enhanced tolerance to oxidative stress caused by the application of methyl viologen (MV, paraquat), a ROS-generating non-selective herbicide. SSA sweetpotato plants showed higher tolerance to chilling stress than non-transgenic (NT) plants, whereas SSA potato plants showed higher tolerance to high temperature. SSA sweetpotato plants showed a strong tolerance to sulfur dioxide (500 ppb). In addition, transgenic potato plants expressing *Arabidopsis* nucleoside diphosphate kinase 2 (NDPK2) gene in cytosols under the control of *SWPA2* promoter (SN plants) showed a significantly enhanced tolerance to MV, high temperature and salt stress compared to NT plants.

Media summary

Molecular breeding by gene manipulation of antioxidative mechanism will result in development of industrial transgenic crops with enhanced tolerance to environmental stresses for sustainable agriculture.

Key Words

Oxidative stress, superoxide dismutase, ascorbate peroxidase, nucleoside diphosphate kinase 2, stress-inducible promoter, molecular breeding, sweetpotato, potato, tall fescue

Introduction

The dramatic increase in population accompanied by rapid industrialization in developing countries has caused imbalances in the supply of food and energy. To cope with these global crises over food and energy supplies as well as environmental problems, it is urgently required to develop new crop varieties to be grown in marginal lands including desertification lands by molecular breeding. In particular, cash crops to increase the income of local farmers in marginal areas should be developed. Oxidative stress derived from reactive oxygen species (ROS) is one of the major factors causing injury to plants exposed to environmental stress. In order to develop various transgenic plants with an enhanced tolerance to multiple environmental stresses, we are focusing on the manipulation of antioxidant genes in plant cells. Recently we developed several transgenic crops such as sweetpotato, potato, tall fescue expressing genes of both Cu/Zn superoxide dismutase (CuZnSOD) and ascorbate peroxidase (APX) in the chloroplasts under the control of an oxidative stress-inducible peroxidase (*SWPA2*) promoter (Kim et al. 2003)(referred to SSA plants). Transgenic crops expressing nucleoside diphosphate kinase 2 (NDPK2)(Moon et al. 2003) gene in cytosols under *SWPA2* promoter (SN plants) were also developed. In the presentation, characterization of transgenic plants such as SSA plants and SN plants will be introduced in term of enhanced tolerance to multiple environmental stresses in detail.

Methods

The chimeric gene construct, named pSSA, expressing CuZnSOD and APX in chloroplasts under the control of an oxidative stress-inducible *SWAP2* promoter was used for transformation of sweetpotato (*Ipomoea batatas* L. Lam cv. Yulmi)(Lim et al. 2007), potato (*Solanum tuberosum* L. cv. Atlantic and Superior)(Tang et al. 2006) and tall fescue (*Festuca arundinacea* Schreb. cv. Kentucky-31) (Lee et al. 2007). The SN vector expressing NDPK2 in cytosols under *SWAP2* promoter was used for transformation (Tang et al. 2008). The detail transformation method and characterization of transgenic plants were described in the references.

Results

SSA plants showed enhanced tolerance to oxidative stress caused by the application of methyl viologen (MV, paraquat), a ROS-generating non-selective herbicide both on the level of leaf discs and whole plants. SSA sweetpotato plants showed higher tolerance to chilling stress than non-transgenic (NT) plants (Figure 1), whereas SSA potato plants showed higher tolerance to high temperature. SSA sweetpotato plants showed a strong tolerance to sulfur dioxide (500 ppb).

SN plants also showed a significantly enhanced tolerance to MV, high temperature and salt stress compared to NT plants. NDPK2 activity in SN potato plants following MV treatment well reflected the plant phenotype. APX activity was also increased in MV-treated SN plants compared to EN plants under the control of CaMV 35S promoter and NT plants (Figure 2).

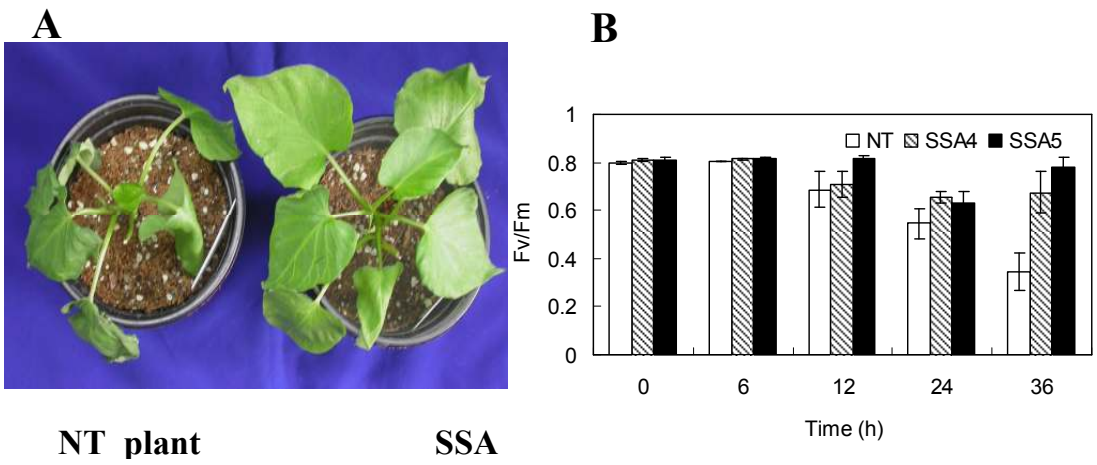

Figure 1. Effect of chilling stress at 4°C on NT and SSA plants. A, Visible differential damages in the leaves of NT and SSA4 plants at 24 h after chilling treatment and at 12 h of recovery at 25°C following chilling. B, Photosynthetic activity (Fv/Fm) in the leaves of NT and SSA plants at 24 h after chilling treatment and at 12 h of recovery at 25°C following chilling.

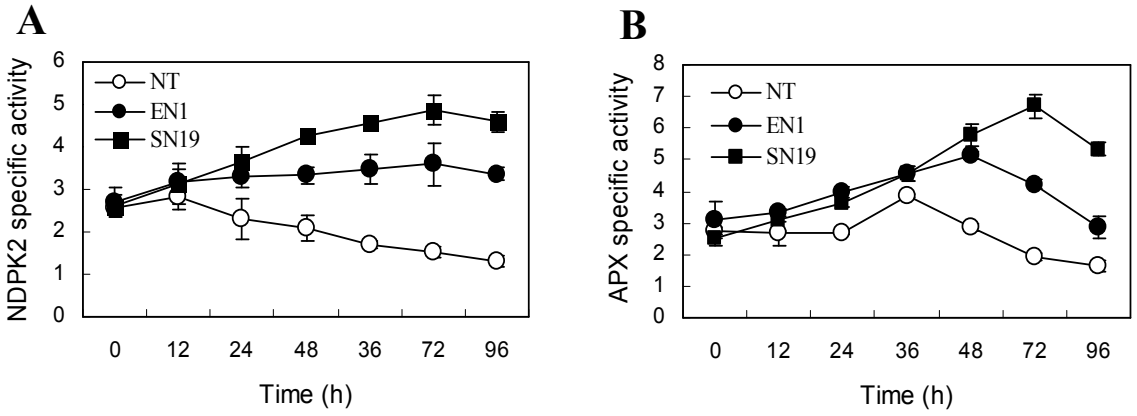

Figure 2. NDPK (A) and APX (B) activities in leaves from NT, EN, and SN plants subjected to 150 μM MV spray. Protein was extracted from leaves 12, 24, 36, 48, 72, and 96 h after treatment with MV. NT, non-transgenic potato plants; EN1 and SN19, transgenic potato plants.

Conclusion

We successfully generated transgenic crops such as sweetpotato, potato and tall fescue plants expressing both Cu/ZnSOD and APX in chloroplasts under the control of an oxidative stress-inducible peroxidase (*SWPA2*) promoter. Transgenic potato plants expressing AtNDPK2 in cytosols under *SWPA2* promoter or constitutive CaMV 35S promoter were also generated. All transgenic plants showed an enhanced tolerance to MV-mediated oxidative stress and abiotic stresses such as extreme temperature and salt stress. Our results strongly suggested that the rational manipulation of antioxidative mechanism will be applicable for the development of all plant species with enhanced tolerance to multiple environmental stresses in marginal lands for sustainable agriculture.

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