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Original Paper

Improvement of environmental stress tolerance of sweet potato by introduction of genes for spermidine synthase

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Abstract With the purpose of enhancing environmental stress tolerance of sweet potato (*Ipomoea batatas*, cv. Kokei 14), we transformed this plant with spermidine synthase genes derived from *Cucurbita ficifolia* (*FSPD1*). The *FSPD1*-transgenic plants showed twice as high spermidine content as the wild type (WT) counterpart in both leaves and storage roots. One of the most characteristic features of the transgenic plants was the increase in the number of storage roots formed under both non-stress and stressful environments. Salt and drought stresses suppressed storage root growth, but the transgenic plants were less affected producing significantly larger mass of storage roots and starches than WT plants under either stress. The transgenic plants also showed increased tolerance to chilling- and heat-mediated damage to photosynthesis compared to the WT plants. Thus, sweet potato was made more tolerant to environmental stresses through introduction of the *FSPD1* genes. This improved tolerance may involve enhanced oxidative stress tolerance at least partially because the transgenic plants were more tolerant to paraquat, a potent oxidative stress inducer, than the WT plants. From these results, the *FSPD1* gene is considered useful for gene transfer technology aiming at improving environmental stress tolerance of sweet potato.

Key words: Polyamine, spermidine synthase gene, stress tolerance, sweet potato.

Crop productivity of agricultural and industrial plants is influenced by the interactions of their inherent eco-physiological traits with physical, chemical and biological environments under which they grow. Diseases, insects, weeds, and adverse climatic and soil conditions are the major environmental impacts limiting crop productivity. According to Boyer (1982), the average crop yield in USA was often substantially reduced as compared with the maximum yield that can be achieved under non-stressful ideal environments, mainly due to unfavorable abiotic environments such as salinity, drought, and high temperature. The recent global warming tendency could accelerate worldwide the frequent occurrence of such stressful environments. Thus, it is crucial to develop crop plants with increased tolerance to various environmental stresses.

Recently, gene transfer approaches have been employed to improve the stress tolerance of plants (Allen 1995; Holmberg and Bülow 1996). A number of attempts have shown the enhanced tolerance to extreme environments through introduction of genes encoding antioxidant enzymes, those involved in modification of membrane lipids and in the biosynthesis of osmoprotectants, late embryonic abundant proteins, ion transporters, transcription factors, and polyamines (Kakkar and Sawney 2002; Shinozaki et al. 2003).

Polyamines, such as spermidine (Spd, a triamine) and spermine (Spm, a tetraamine) and their obligate precursor putrescine (Put, a diamine), are ubiquitous cellular constituents and play important roles in various physiological and developmental processes. Many of the physiological functions of polyamines in the cell have been attributed to their polycationic nature at physiological pH, which enables them to interact with essential molecules such as nucleic acids, proteins and membrane phospholipids, thereby activating or stabilizing these molecules (Evans and Malmberg 1989). In plants, Put is synthesized via the two main pathways,

Abbreviations: ADC, arginine decarboxylase; CaMV, cauliflower mosaic virus; 4FA, 4-fluorophenoxyacetic acid; LS, Linsmaier and Skoog; ODC, ornithine decarboxylase; PPFD, photosynthetic photon flux density; Put, putrescine; ROS, reactive oxygen species; SAM, S-adenosylmethionine; SAMDC, S-adenosylmethionine decarboxylase; Spd, spermidine; SPDS, spermidine synthase; Spm, spermine; WT, wild type.

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i.e. ornithine decarboxylase (EC 4.1.1.17, ODC) and arginine decarboxylase (EC 4.1.1.19, ADC) pathways (Bagni and Tassoni 2001). Spd and Spm are synthesized from Put by the addition of aminopropyl moiety to one and both amino groups of Put in a reaction catalyzed by Spd synthase (EC 2.5.1.16, SPDS) and Spm synthase (EC 2.5.1.22), respectively. Aminopropyl moiety is donated from decarboxylated S-adenosylmethionine (SAM), which is derived from SAM in a reaction catalyzed by SAM decarboxylase (EC 4.1.1.50, SAMDC). Genes encoding these polyamine biosynthetic enzymes have been cloned and characterized from different plant sources (Malmberg et al. 1998).

A number of physiological studies have indicated important roles of polyamines in plant defense to a wide array of environmental stresses (Bouchereau et al. 1999; Shen et al. 2000; Bais and Ravishankar 2002; He et al. 2002). Now, several gene transfer approaches have shown that the plants overexpressing ADC, ODC or SAMDC transgenes from different sources exhibit enhanced tolerance to salt and drought stresses (Roy and Wu 2001, 2002; Kumria et al. 2002; Mo and Pua 2002; Capell et al. 2004). In addition, we found recently that *Arabidopsis thaliana* overexpressing *SPDS* transgenes from *Cucurbita ficifolia* was highly tolerant to multiple environmental stresses including chilling and freezing temperatures, salinity, drought, hyperosmosis, and paraquat toxicity, as compared with the wild type (WT) counterpart (Kasukabe et al. 2004). Since crop plants may often encounter several different kinds of environmental stress simultaneously, genes for SPDS could play an important part in the strategies to improve stress tolerance of crop plants by using gene transfer technology.

Sweet potato [*Ipomoea batatas* (L.) Lam.] is a Convolvulacea root crop native to tropical Americas and cultivated worldwide as a valuable source of food, animal feed and industrial raw material. China is a world's largest sweet potato production country, producing about 105 million tons (83% of the world harvest) on 4.9 million hectares in 2004. The main target of sweet potato breeding in China and other countries is to improve the environmental stress tolerance besides resistance to diseases, insects and herbicides (Yushi and Watanabe 2004). Because of the biological complexities of sweet potato, sexual hybridization strategies have not been very effective in developing improved sweet potato cultivars (Prakash 1994). Thus, in this communication we examined the possibility that the gene transfer technology using the gene encoding SPDS could be useful for improving tolerance of sweet potato against various environmental stresses.

Materials and methods

Plant material

Sweet potato (cv. Kokei 14) was used as the plant material. The embryonic callus was induced from the shoot meristem incubated at 26°C in the dark on the embryonic callus induction medium. The medium consisted of LS medium (Linsmaier and Skoog 1965) supplemented with 1 mg l^{-1} 4-fluorophenoxyacetic acid (4FA), 30 g l^{-1} sucrose and 3.2 g l^{-1} gellan gum and adjusted to pH 5.8 (Otani and Shimada 1996). After about 2 months, light yellow calli were isolated and used for transformation.

Transformation and regeneration of transgenic plants

The gene used for transformation of sweet potato was the *SPDS* gene isolated from a cDNA library prepared from *C. ficifolia* and fused with the CaMV-35S promoter (Kasukabe et al. 2004). The gene, designated as *FSPD1* (GenBank accession No. BD142348), shares 84% homology in the deduced nucleotide sequence with *Arabidopsis* SPDS cDNA (Hashimoto et al. 1998).

Transformation of the embryonic callus was performed as described by Otani et al. (1998). *Agrobacterium tumefaciens* strain EHA101 harboring the binary vector plasmid pBI101-*35S:FSPD1*(+)-Hm2 was grown on LS medium supplemented with 50 mg l^{-1} kanamycin, 50 mg l^{-1} hygromycin and 15 g l^{-1} agar for two days. Then, the bacterial colony was suspended in liquid LS medium supplemented with 10 mg l^{-1} acetosyringone, 1 mg l^{-1} 4FA, and 30 g l^{-1} sucrose, and shaken at 100 rev. min^{-1} for 1 h. The embryonic calli were soaked in the bacterial suspension for 2 min and transferred to LS medium supplemented with 1 mg l^{-1} 4FA, 10 mg l^{-1} acetosyringone, 30 g l^{-1} sucrose and 3.2 g l^{-1} gellan gum. After three days, the infected calli were washed three times with sterile distilled water containing 500 mg l^{-1} carbenicillin, and transferred to LS medium supplemented with 1 mg l^{-1} 4FA, 25 mg l^{-1} hygromycin, 500 mg l^{-1} carbenicillin, 30 g l^{-1} sucrose and 3.2 g l^{-1} gellan gum. After incubation on this selection medium for sixty days with a change of medium every two weeks, light yellow calli were selected and used for plant regeneration. All of the above cultures were carried out at 26°C in the dark and at the medium pH of 5.8.

For plant regeneration, the selected calli were transferred onto LS medium supplemented with 4 mg l^{-1} abscisic acid, 1 mg l^{-1} gibberellic acid (GA_3), 25 mg l^{-1} hygromycin, 500 mg l^{-1} carbenicillin, 30 g l^{-1} sucrose and 3.2 g l^{-1} gellan gum (pH 5.8), and incubated at 26°C under continuous light. Fluorescent tubes (Plantlux, Toshiba, Japan) provided light with a photosynthetic

photon flux density (PPFD) of 40 μmol m^{-2} s^{-1} at a plant level. After two weeks, the calli were transferred to LS medium supplemented with 0.05 mg l^{-1} GA_3, 25 mg l^{-1} hygromycin, 500 mg l^{-1} carbenicillin, 30 g l^{-1} sucrose and 3.2 g l^{-1} gellan gum (pH 5.8). They were grown under the same temperature and light conditions until somatic embryos were formed from the hygromycin-resistant calli. Then, the green-colored calli with somatic embryos were transferred to LS medium supplemented with 30 g l^{-1} sucrose and 3.2 g l^{-1} gellan gum (pH 5.8) and incubated with a change of medium at two-week intervals until plants were regenerated. We obtained seven transgenic plants (lines TSP-SS-1 to and TSP-SS-7) regenerated from different calli.

Regenerated transgenic plants and vine cuttings of WT plants were planted into plastic containers filled with 20 l of commercially available nursery soil (Sansanshodo, Takii Seeds Co., Kyoto, Japan). The containers were placed in the growth chamber kept at 25/22°C (day/night), 55% RH and a PPFD of 40 μmol m^{-2} s^{-1} for about two weeks. Then, they were moved to the glasshouse and the plants were grown at 25/22°C and 55% RH under natural light. These plants served as mother plants for propagation by means of vine cuttings with two leaves on the main stem. One lateral shoot was allowed to elongate and when it expanded five leaves, they were used for stress tolerance assessment.

PCR and immunoblot analyses

For PCR analysis, genomic DNAs were extracted from young leaves of about 0.4 g fresh weight with Plant DNAzol reagent (Life Technologies, U.S.A.) according to the manufacture's instructions. The primers used for amplification of a 860-bp fragment of the CaMV-35S promoter and *FSDD1* genes were primer 1 [35SPCR-1(5′-GTGATATCTCCACTGACGTA-3′)] and primer 2 [SPDR-2(5′-ACACAACGCCTCCTGGTCGAAGAGC-3′)]. The conditions for PCR were 30 cycle of 92°C for 1 min and 58°C for 2 min and 72°C for 2 min. The products were separated by electrophoresis on a 1.5% (w/v) agarose gel, stained by ethidium bromide and visualized under ultraviolet light.

For immunoblot analysis, leaves of about 0.2 g fresh weight were homogenized with a Polytron in 1 ml of ice-chilled buffer containing 10 mM Tris-HCl (pH 8.0), 2 mM dithiothreitol and 0.2 mM *p*-mercuribenzoate. The homogenate was centrifuged at 27,000×*g* for 20 min at 4°C. Proteins in the supernatant were quantified by the method of Bradford (1976) with bovine serum albumin as the standard. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis using a Ready Gel J gel (Bio-Rad) by the method of Laemmli (1970), and electroblotted onto polyvinylidene difluoride membranes (Sequi-blot PVDF membrane, Bio-Rad). Membranes were incubated with the *FSPD1* antibody raised in rabbit against a peptide corresponding to the 14 amino acids (LCSTEGPPLDFKHP) of the *FSPD1* C terminus. Immunoreactive proteins were detected with a second antibody, goat anti-rabbit IgG (H+L) (Human IgG Adsorbed) horseradish peroxidase (Bio-Rad).

Chemical analysis

For quantifying polyamines, fresh plant materials (leaves and storage roots) were homogenized in 0.5 M perchloric acid using an ice-chilled Polytron homogenizer. After centrifugation, free polyamines in the supernatant were dansylated with dansyl chloride (10 mg ml^{-1} acetone) and quantified via HPLC with 1,6-hexanediamine as the internal standard, as described by Song et al. (2002). Chlorophylls in leaves were extracted with 80% (v/v) acetone and quantified spectrophotometrically by the method of Arnon (1949).

Salt and drought stress tolerance assessment

Rooted cuttings of transgenic (lines TSP-SS-1 and TSP-SS -4) and WT plants were transplanted into plastic containers (four plants/container) filled with 20-l nursery soil supplemented with 13.4 g of a polymer-coated commercial fertilizer (Ekorongu, $N : P_2O_5 : K_2O = 14 : 12 : 14$, Zen-no, Tokyo, Japan) and 3.6 g K_2SO_4, and grown in the glasshouse kept at 25/22°C (day/night) and 55% RH under natural light during summer. Saline soil was established by the addition to the 20-l soil of 8 g NaCl before planting and again 4 g NaCl 45 days after planting. Plants in salt stress and non-stress (control) treatments were irrigated with deionized water when the soil water tension increased to pF 2.3 until it decreased to pF 1.5 (field capacity). Plants in drought stress treatment were irrigated in the same way as above during the first week after planting, after which irrigation was commenced at pF 2.9 and ended at pF 2.2. Each treatment consisted of two replications. Vines were trained vertically by using a training rod. Plants were harvested 114 days after planting, and fresh weights of vines and storage roots and polyamine contents in the fifth leaves from the top and the storage roots were determined.

Weak light treatment

Rooted cuttings of transgenic (lines TSP-SS-1, TSP-SS-2 and TSP-SS-4) and WT plants (15–28 each) were grown in the growth chamber controlled at 25/22°C (day/night), a 16-h photoperiod and a PPFD of 40 μmol m^{-2} s^{-1} from fluorescent tubes. After cultivation for 3 months, the formation of storage roots was determined.

Chilling and heat tolerance assessment

Rooted cuttings of transgenic (lines TSP-SS-1 and TSP-SS-4) and WT plants were transplanted into nursery soil

fertilized as above and grown in the glasshouse kept at 28/23°C (day/night) and 60% RH under natural light during summer. When the plants formed 22 to 25 expanded leaves, leaf discs of 1.5 cm in diameter were cut from the 8th to 12th leaves from the top. In one experiment, leaf discs were placed in a Petri dish lined with wet filter paper and exposed to 10, 15, 20, and 30°C for 6 hours under a moderate light of 240 μmol m^{-2} s^{-1} PPFD (chilling treatment). Immediately thereafter, the net photosynthetic rate (O_2 evolution rates in the light) was measured in a Clark-type oxygen electrode (Rank Brothers, Cambridge, UK) at 25°C and a PPFD of 1050 μmol m^{-2} s^{-1} as described previously (He et al. 2002). The net photosynthetic rate of leaf discs that were not subjected to chilling treatment was used as the control. In another experiment, leaf discs were dipped in hot water held at 42, 45, and 47°C for 5 minutes in the dark (heat treatment). The net photosynthetic rate was measured in the same way as described above.

Paraquat tolerance assessment

Tolerance to paraquat (methyl viologen), a well-known oxidative stress inducer (Calderbank 1968), was assessed in terms of the capacity of leaves to withstand oxidative stress-induced chlorophyll degradation. Leaf discs prepared from transgenic (lines TSP-SS-1 and TSP-SS-4) and WT plants as described above were floated on 0.01% (v/v) Tween 20 solution supplemented with paraquat at 0–10 μM and incubated at 25°C and a PPFD of 240 μmol m^{-2} s^{-1} for 38 hours. Then, the chlorophyll content was determined as described above.

Results

Analysis of the 35S:FSPD1 plants

The second unfolded leaves from the top of the growth chamber-grown transgenic and WT plants were used for molecular analysis. PCR analysis revealed the presence of the introduced gene in all of the seven transgenic plant lines, showing a clear single signal at the 860-bp position, which was not observed in WT plants (Figure 1A). The size of the amplification fragment was the same as that of the introduced gene (PC), confirming that all lines of transgenic plants we obtained had successfully incorporated the *FSPD1* gene. Immunoblot analysis with the antibody raised against *C. ficifolia* SPDS revealed the expression in transgenic plants of an immunoreactive protein of 35.5 kDa (Figure 1B), which corresponded, in terms of molecular mass, to soybean SPDS (Yoon et al. 2000). The phenotypes of transgenic plants were not greatly different from those of WT plants, but the internode was characteristically shorter in the former.

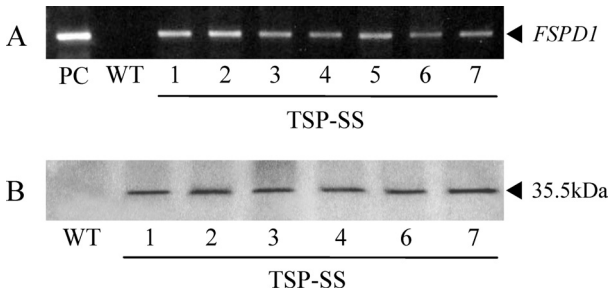

Figure 1. The *FSPD1* transgene insertion in genomic DNA (A) and its translation (B) in the leaves of the wild type (WT) and *FSPD1*-transgenic (TSP-SS) plant lines of sweet potato. PC in panel A represents the *FSPD1* plasmid as a positive control.

Polyamine content and tolerance to salt and drought stresses

Figure 2 depicts polyamine contents in leaves and storage roots of transgenic (lines TSP-SS-1 and TSP-SS-4) and WT plants that were subjected to salt, drought, and control treatments. In the control treatment, both transgenic plant lines showed 1.5- and 2-fold increases in

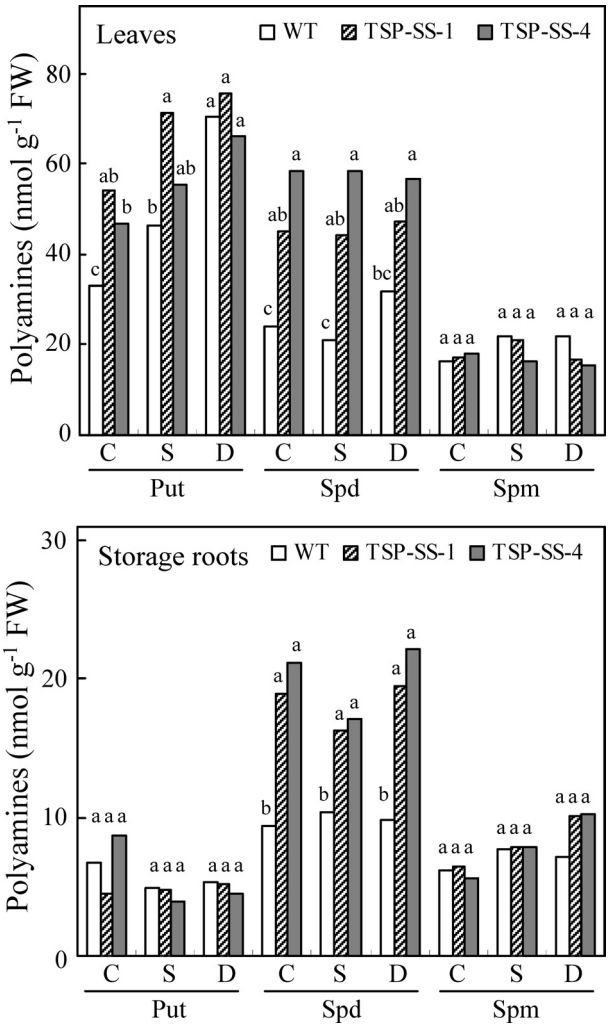

Figure 2. Polyamine content in leaves and storage roots of the wild type (WT) and *FSPD1*-transgenic (TSP-SS) plant lines of sweet potato as influenced by salt and drought stress in the root zone. For growth conditions see text. Means with different letters within a polyamine species are significantly different at $P<0.05$ by Turkey's multiple range test. C: control, S: salt stress, D: drought stress.

Table 1. Effects of salt and drought stress on plant growth and starch content of storage root in wild type (WT) and *FSPD1*-transgenic (TSP-SS-1 and -4) sweet potato

	Plant	Growth condition		
		Control	Salinity	Drought
Fresh weight of vines (g plant^{-1})	WT	249.6 a	338.6 a	167.3 b
	TSP-SS-1	249.4 a	274.8 a	144.1 b
	TSP-SS-4	257.3 a	258.6 a	144.0 b
No. of storage roots per plant	WT	3.3 cd	4.0 c	2.7 d
	TSP-SS-1	5.0 ab	6.1 a	4.5 bc
	TSP-SS-4	5.0 ab	5.3 ab	4.0 c
Fresh weight of storage roots (g plant^{-1})	WT	324.0 a	237.1 c	111.3 d
	TSP-SS-1	348.9 a	284.0 b	139.1 d
	TSP-SS-4	338.2 a	284.3 b	127.2 d
Starch content in storage roots (%, w/w)	WT	15.0 b	16.7 ab	12.4 c
	TSP-SS-1	15.1 b	17.0 ab	15.2 b
	TSP-SS-4	15.7 b	17.5 a	17.1 ab
Starch yield (g/plant)	WT	48.6 a	39.6 b	13.8 d
	TSP-SS-1	52.7 a	48.3 a	21.1 c
	TSP-SS-4	53.1 a	49.8 a	21.8 c

Means with different letters are significantly different at $P<0.05$ by Turkey's multiple range test.

Put and Spd contents in the leaves, respectively, as compared with WT plants. Under salt and drought stress, Put content increased in both of transgenic and WT plants, particularly in WT plants under drought stress. Spd and Spm contents were unaffected by either salt or drought stress, except for Spd in WT plants that increased considerably under drought stress. On the other hand, storage roots of transgenic plants, under control conditions, showed about twice as high Spd content as those of WT plants. Put and Spm contents did not differ between them. Salt stress did not affect polyamine content in storage roots but drought stress increased Spm content in those of transgenic plants. Differences in polyamine patterns between the two lines of transgenic plants were small under both control and stress conditions with some exceptions.

The effects of salt and drought stresses on plant growth and starch contents of storage roots were slightly but significantly different between the transgenic and WT plants (Table 1, Figure 3). In the control treatment, growth rates of vines and storage roots as well as starch content in storage roots did not differ between the transgenic and WT plants, with the exception that the number of storage roots formed was larger in the former. Under salt stress, both transgenic and WT plants showed a decrease in fresh weights of storage roots with a slight increase in the number of storage roots formed, as compared with the control. It should be noted here that transgenic plants had about 1.2-fold larger fresh mass of storage roots than WT plants. Starch content in storage roots increased under salt stress, which was slightly more pronounced in transgenic plants. As a consequence, starch yield per plant was not greatly different between the control and salt stress treatments in transgenic plants, while in WT plants it was reduced by about 20% under salt stress. Salt stress did not affect vine growth in transgenic plants, while it increased fresh weights of vines in WT plants despite insignificantly. On the other hand, drought stress caused a marked growth reduction in both vines and storage roots. Here also, transgenic plants were less affected than WT plants in terms of the number of storage roots formed and their fresh weights. Drought stress decreased starch content of storage roots in WT plants but not in transgenic plants, resulting in a 1.5-fold higher starch yield in transgenic plants than in WT plants. Thus, the *FSPD1*-transgenic sweet potato was more tolerant to both salt and drought stresses in the root zone than the WT counterpart. Physical properties of starch powder such as peak paste viscosity and a temperature at which the peak viscosity is attained were not different between transgenic and WT plants and unaffected by the environmental conditions under which they were grown (data not shown).

Storage root formation under a weak light

Light is one of the major environmental factors that affect storage root initiation and growth in sweet potato (Kays et al. 1992). Reducing the light intensity to 30% of full sunlight markedly inhibits storage root growth (Martin 1985), although the critical light intensity for the storage root initiation is unknown. Thus, we surveyed the ability of the *FSPD1*-transgenic sweet potato to form storage roots under very weak light conditions. Figure 4 shows the formation of storage roots in transgenic (TSP-SS-1, -2 and -4) and WT plants grown at a PPFD of 40 μmol m^{-2} s^{-1} for three months. WT plants did not form any storage roots, while some transgenic plant lines formed one or two small storage roots (10–20 g fresh weight). The percentage of plants forming the storage root was 33 to 89% depending on the lines. The fresh mass of fine roots was smaller in transgenic plants than in WT plants. Vine growth did not differ between them.

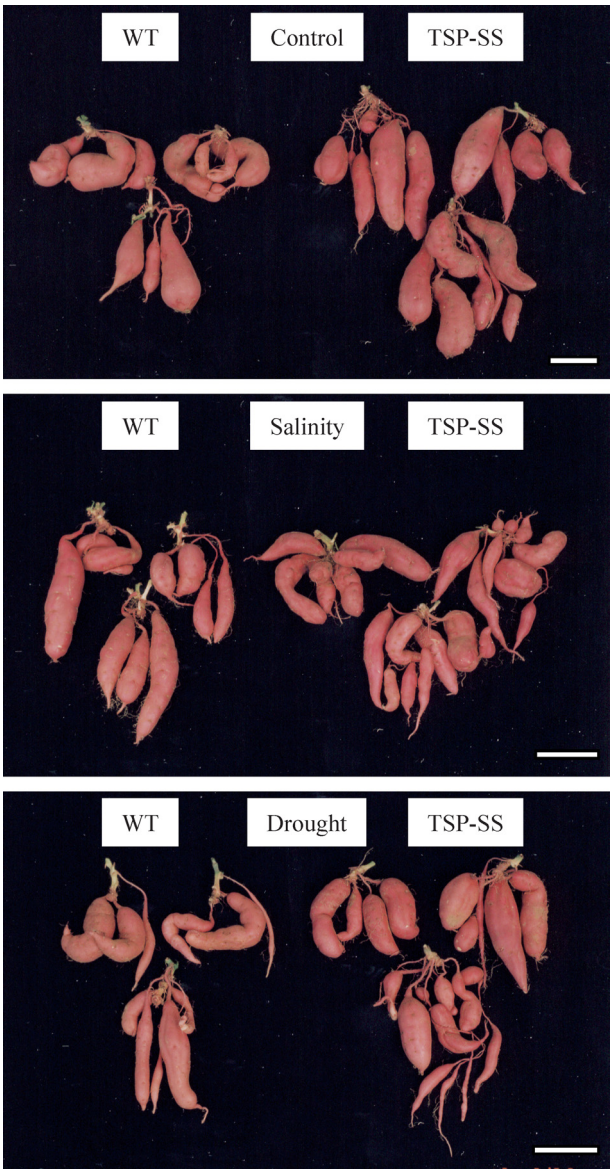

Figure 3. Storage root formation in the wild type (WT) and *FSPD1*-transgenic (TSP-SS) plant lines of sweet potato grown in the glasshouse under control, saline and dry soil conditions. For growing conditions see text. The photograph was taken 114 days after planting. Scale bar=50 mm.

Similar experiments were repeated once with almost identical results.

Chilling and heat stress tolerance of photosynthesis

Figure 5 depicts the effects of chilling (Figure 5A) and heat (Figure 5B) treatments on the photosynthesis of transgenic (lines TSP-SS-1 and TSP-SS-4) and WT plants. In the chilling treatment, upon exposure of leaf discs to 10°C, the photosynthetic activity was decreased to 33% of control in WT plants, while it was decreased to 70% of control in transgenic plants (Figure 5A). In the heat treatment, leaf discs of WT plants lost 25% of control activity after exposure to 45°C, whereas this level

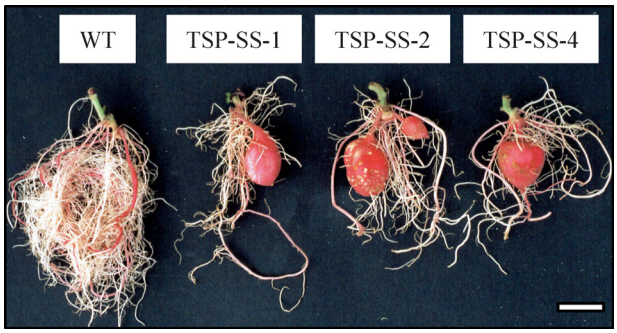

Figure 4. Storage root formation under a weak light in the wild type (WT) and *FSPD1*-transgenic (TSP-SS) plant lines of sweet potato. Rooted cuttings were planted in soil-filled pots and grown in the growth chamber kept at 25/22°C (day/night), a 16-h photoperiod and a PPFD of 40 μmol m^{-2} s^{-1}. The photograph was taken three months after planting. Scale bar=25 mm.

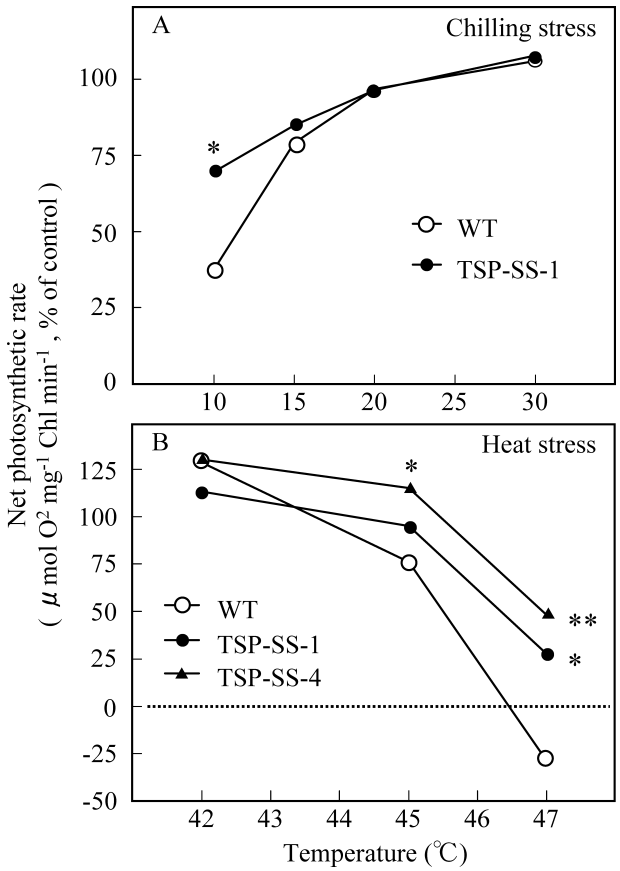

Figure 5. Chilling and heat tolerance of photosynthesis in the wild type (WT) and *FSPD1*-transgenic (TSP-SS) plant lines of sweet potato. For the experimental methods see text. The net photosynthetic rate in stressed leaves is expressed in percent of that in unstressed leaves (control). The control rates in WT and transgenic plants were 0.62–0.67 and 1.03–1.17 μmol O_2 mg^{-1} Chl min^{-1}, respectively. The single and double asterisks indicate the significant difference between the WT plant and a transgenic plant line at $P<0.05$ and 0.01, respectively, by Student's t-test.

of heat stress caused little damage to photosynthesis in transgenic plants (Figure 5B). When the temperature was increased to 47°C, the rate of net photosynthesis in WT

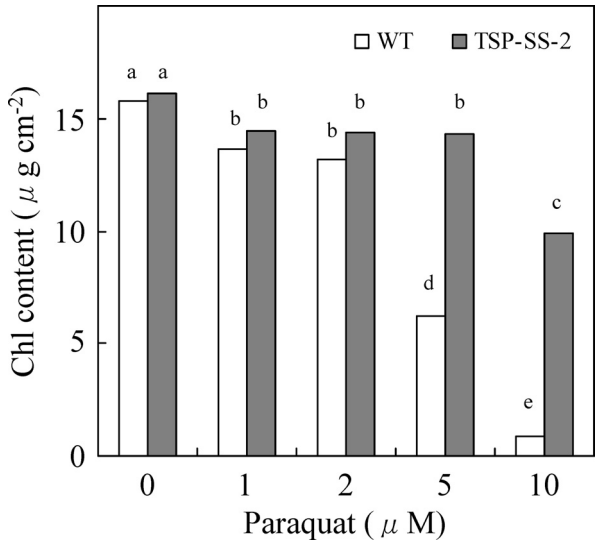

Figure 6. Tolerance to paraquat-mediated chlorophyll degradation in leaves of the wild type (WT) and *FSPD1*-transgenic plant line (TSP-SS-2) of sweet potato. Means with different letters are significantly different at $P<0.05$ by Turkey's multiple range test.

plants decreased to a negative value, mainly due to a decline of the photosynthetic O_2 evolution rate below the respiratory O_2 consumption rate. However, transgenic plants showed the positive rate of net photosynthesis although the rates were reduced to 28 and 48% of control rate in TSP-SS-1 and -4, respectively. Thus, the *FSPD*1-transgenic sweet potato was more tolerant to both chilling- and heat-mediated damage to photosynthesis than the WT counterpart. Interestingly, in both experiments the transgenic plants showed about 1.6-fold higher photosynthetic rates than WT plants under control conditions (see the footnote to Figure 5).

Tolerance to paraquat toxicity

As shown in Figure 6, leaf discs of WT plants showed a significant loss of chlorophyll after exposure to 5 μM paraquat, and at 10 μM paraquat chlorophyll content decreased to almost zero. On the other hand, leaf discs of transgenic plants (TSP-SS-2) retained 70% of original chlorophyll content after exposure to 10 μM paraquat, indicating the increase in tolerance to paraquat-induced oxidative stress in the *FSPF1*-transgenic sweet potato.

Discussion

Under control glasshouse conditions, the *FSPD1*-transgenic sweet potato showed about a 2-fold increase in foliar Spd content over the WT counterpart, indicating the up-regulation of SPDS activity in transgenic plants. This result agrees with previous ones with *FSPD1*-overexpressing *Arabidopsis* (Kasukabe et al. 2004) and transgenic tobacco harboring *Datura SPDS* transgenes (Franceschetti et al. 2004). The *FSPD1*-transgenic plants also showed higher Put content in the leaves than the WT plants. This increase of Put could be ascribable to enhanced interconversion of Spd into Put through the acetylation mechanism (De Agazio et al. 1995), possibly as a result of increase in the endogenous Spd content. However, the transgenic plants grown in the growth chamber under a weak fluorescent light (40 μmol m^{-2} s^{-1} PPFD) did not show any increases in Put content over the WT plants (data not shown). This may suggest the versatility of polyamine metabolism in the *FSPD1*-transgenic sweet potato in response to light conditions.

The transgenic plants showed a similar rate of vine growth to the WT plants under control glasshouse conditions, but the number of storage roots formed was significantly larger in the former. In addition, the transgenic plants were capable of forming storage roots even under a very weak light conditions, under which the WT plants formed fine roots alone. Furthermore, during the course of this study, we came upon the *FSPD1*-transgenic plants with a massive increase in the number of storage roots formed (data not shown). Although many of the individual storage roots were small, their total fresh mass was about 1.5-fold larger than that in WT plants. It is well known that storage root formation in sweet potato depends on the cell division activity of the primary cambium between the protophloem and protoxylem and the secondary meristems in the xylem in particular (Wilson and Lowe 1973). Recently, Tanaka et al. (2005) observed a considerable increase in the expression of several genes during storage root formation in sweet potato, some of which encode proteins involved in signal transduction including protein kinases. It is well documented that polyamines promote cell division (Evans and Malmberg 1989). Furthermore, polyamines may play important roles in signal transduction pathways in both animal and plant cells probably through activation of protein kinases and transcription factors (Datta et al. 1987, Sudha and Ravishankar 2002, Childs et al. 2003). Previously, we found a substantial increase, during during, in the expression of genes for protein kinase-like protein and several transcription factors like WRKY, DREB and CONSTANS B-box zinc finger protein in *Arabidopsis thaliana* harboring the *FSPD1* transgenes (Kasukabe et al. 2004). In the present study, the *FSPD1*-transgenic sweet potato had twice as high Spd content in storage roots as did the WT plants. Thus, we hypothesize that the marked increase of Spd content in the roots could have played a role in enhancing the potential for storage root formation in the *FSPD1*-transgenic sweet potato. Further study is needed to substantiate this hypothesis. Concerning the higher photosynthetic rate in transgenic plants than in WT plants under control conditions, the increased formation of storage roots in the former plants could be a cause. This is because photosynthesis is often

stimulated by the increase in the rate of assimilate import by sink organs like storage roots (Spence and Humphries 1972).

As compared with the WT counterpart, the *FSPD1*-transgenic sweet potato exhibited enhanced tolerance to both salt and drought stresses in terms of storage root growth. Also, the transgenic plants were more tolerant to chilling- and heat-mediated damage to photosynthesis than the WT plants. Based on these results, we conclude that the *FSPD1*-transgenic sweet potato has acquired enhanced tolerance to environmental stresses. The ability of the transgenic plants to develop storage roots under chilling and heat stresses remains to be determined.

A major cause of stress-induced damage to plants has been associated with a remarkable increase in generation of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and hydroxyl radicals (Allen 1995, Zhu 2001). Plants with high antioxidant enzyme activities are generally more tolerant to various environmental stresses than those with low enzyme activities. It is well documented that polyamines have a ROS-scavenging property (Løvaas 1997, Das and Misra 2004). Furthermore, polyamines can induce an increase in antioxidant enzyme activities and thereby control free radical generation in plants during exposure to environmental stress (Verma and Mishra 2005). We found previously that *FSPD1*-transgenic *Arabidopsis thaliana* showed a greater increase in activities of antioxidant enzymes such as superoxide dismutase and ascorbate peroxidase in leaves in response to chilling than the WT counterpart (He et al. 2003). In the present study, the transgenic plants were more tolerant to ROS-generating paraquat than the WT plants. In addition, the transgenic plants showed much lower hydrogen peroxide concentrations in chilled leaf discs than the WT plants (data not shown). Paraquat is known to divert electrons from photosystem I to molecular oxygen, generating superoxide anions in chloroplasts (Calderbank 1968). Environmental stresses including chilling, heat and salt stresses may also enhance ROS generation in chloroplasts, thereby causing oxidative damage to the photosynthetic apparatus (Foyer 1997, Holmberg and Bülow 1998). Thus, it is inferred that the *FSPD1*-transgenic sweet potato exhibits higher antioxidant enzyme activities in chloroplasts than the WT plants, which could have contributed at least in part to the enhanced stress tolerance in the transgenics. In animal cells, polyamines may increase the permeability of superoxide dismutase across membranes by covalently binding to the enzyme proteins (Poduslo and Curran 1996). If this holds true in plant cells, then the increased Spd in leaf cells could facilitate the incorporation of cytosolic superoxide dismutase into chloroplasts. Further study is needed to clarify the mechanism by which the increase in cellular Spd concentrations induces enhanced tolerance to environmental stresses in sweet potato.

In summary, the results of the present study corroborate the usefulness of the *FSPD1* genes for gene transfer technology aiming at improving environmental stress tolerance of crop plants and thereby reducing the loss of crop production due to adverse climatic and soil conditions.

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