Superoxide Dismutase and Stress Tolerance of Four Tomato Cultivars

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Abstract. Effects of water stress on superoxide dismutase (SOD) activities, changes in protein content, leaf water potential (Ψ_l) and growth were studied in drought-sensitive Kyokko (KK) and Ratan (RT), and drought-tolerant TM0126 (TM) and VF-134-1-2 (VF) cultivars of tomato (*Lycopersicon esculentum* Mill.) in order to obtain fundamental information for breeding drought tolerant cultivars that may be adapted to water stress in many parts of the world. Growth of drought-tolerant TM and VF was greater than that of drought-sensitive KK and RT under water stress conditions. Leaf water potential (Ψ_l) decreased by water stress treatments in all the cultivars, but the reduction was much more rapid and pronounced in KK and RT than VF and TM. Ψ_l of stressed cultivars decreased by 30% to 40% compared to the untreated control cultivars. The initial reduction in the range of 20% to 35% was more rapid in KK and RT than TM and VF. SOD activities were increased by water stress in all cultivars. Increase of SOD activities by water stress was much more rapid and pronounced in TM and VF than in KK and RT. Leaf protein concentration was decreased by the water stress treatments in all cultivars evaluated. In KK and RT, much more rapid reductions in protein concentration were observed than in TM and VF. The regression analysis of Ψ_l and SOD suggest the possibility to using SOD activities as an additional screening criterion for tomato drought tolerance improvement.

Superoxide dismutase (SOD; E.C. 1.15.1.1) is considered to be vital for survival and function of aerobic organisms. These metalloenzymes scavenge the superoxide radicals that are constantly formed as by-products of aerobic metabolism. In plants the photosynthetic process produces high intra-cellular concentrations of active oxygen species and reduced compounds such as NADPH (Asada and Takahashi, 1987). This process must therefore be accompanied with a protective array of enzymes and antioxidants that prevents membrane peroxidation and other types of

injury. Oxyradicals are also formed in cellular compartments other than the chloroplast (Elstner, 1982; Halliwell, 1984); thus SODs are considered to be important components of the defense mechanisms in plants and have consequently been the focus of many research activities. In the presence of metal ions (such as iron), SOD and hydrogen peroxide can react in a Haber-Weiss reaction to form hydroxyl radicals:

This enzyme is unique in that its activity determines the concentrations of $\text{O}_2^{\cdot-}$ and H_2O_2 , the two Haber-Weiss reaction substrates, and it is therefore likely to play a central role in the defense mechanism for plants.

A plant response to drought stress is a complex phenomenon that appears to involve the synthesis of polyamines and a new set of proteins whose function is largely unknown (Caplan et al., 1990). Drought also reduces

the availability of CO_2 for photosynthesis, which can lead to the formation of reactive oxygen species from misdirecting electrons in the photo systems. Hence, mechanisms that reduce oxidative stress may play an important secondary role in drought tolerance (Bowler et al., 1992).

In tomato (*Lycopersicon esculentum* Mill.), cytosolic Cu/Zn SOD is reduced by drought while the chloroplastic Cu/Zn SOD remained largely unaffected (Gianinetti et al., 1993). Glutathione reductase activity increased in drought-stressed wheat (*Triticum aestivum* L.) and cotton (*Gossypium hirsutum* L.) and it was proposed that as well as removing H_2O_2 , this increase may convert NADP that can accept electrons from Ferredoxin to an available form, and thereby minimize superoxide formation. In drought-tolerant *Hordeum* species, levels of glutathione reductase and ascorbate peroxidase increased, but SOD activity was not examined (Smirnoff and Colombe, 1988). Drought-stressed cotton was found to be resistant to a subsequent challenge of paraquat (Burke et al., 1985), which may indicate the existence of a common protective mechanism against these stresses.

Drought-induced changes in lipid peroxidation and the activities of SOD and catalase were compared in two mosses, the drought-tolerant *Tortula ruralis* and the drought-sensitive *Cratoneuron filicinum* (Dhinsa and Motowe, 1981). During stress treatment the drought-tolerant moss showed lower levels of lipid peroxidation, together with increased levels of both enzymes; the opposite occurred in the drought-sensitive moss. Drought tolerant and intolerant maize inbred were analyzed by Malan et al. (1990), and tolerance to drought was found to correlate with Cu/Zn SOD and glutathione reductase activities.

Therefore, increased SOD activities may be directly related to drought tolerance in tomato. In addition, increased SOD activities may be a useful criterion for drought tolerance if stable relationships with tolerance can be identified. Our objective in this study was to analyze the relationship between drought tolerance and SOD activities in four tomato cultivars and to determine if SOD activity is a worthwhile screening criterion for drought tolerance in tomato.

Materials and Methods

Plant materials and growing conditions. Four cultivars of tomato were selected for this study. TM0126 (TM) and VF 134-1-2 (VF) are drought-tolerant and Kyokko (KK) and Ratan (RT) are drought-sensitive cultivars. Seeds of the four cultivars were disinfested with the fungicide Benlate (Dupont Ltd., Wilmington, Del.) for 2 min, soaked in distilled water for 24 h, and sown in a plastic container of vermiculite on 4 Apr. 1997. Benlate was also applied to plants every 7 to 10 d to prevent disease infections. Seedlings were grown in a phytotron under 25 °C day/20 °C night temperature regime. Ten days after sowing, the plants were transferred to plastic pots (9 cm diameter) filled with 1 perlite : 2 sand : 1 peat (by volume) and placed

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under 23 °C day/18 °C night regime. On 21 Apr., plants were retransplanted to 30-cm-diameter pots filled with 1 perlite : 2 sand : 1 peat (by volume) and placed in a plastic greenhouse at Kyoto Univ., Japan under natural temperature conditions (daily maximum temperatures ranging from 26.3 to 35.7 °C and daily minimum temperature 15.4 to 25.6 °C). On 1 May, water stress treatments were initiated when plants had four to five nodes/plant (≈25–30 cm height) in all cultivars. The plants were separated into two groups. Half of the plants received no water, and the remaining plants were well watered. Half strength Enshi nutrient solutions (Yamasaki, 1982) for macro elements and Arnon nutrient solution (Arnon and Hoagland, 1940) for microelements were applied three times a week except during the treatment period. During the treatment period, a slow release granular fertilizer containing 14% of N, 5.28% of P and 9.96% of K was applied to each pot. Plants were sampled at 0, 3, 6, and 9 d after the onset of the water treatments (DAT). Samples were also collected 2 and 4 d after rewatering (DAW). For dry weight measurements, the individual plants were harvested and oven dried at 80 °C for 96 h at the end of the experiment.

Experimental design. A split-plot design was used with four replications. The main plot consisted of the water stress treatments (stress vs. no stress), and the subplot factor was tomato cultivar (four tomato cultivars). Forty tomato plants were used per cultivar per replication. Of the 40 plants, 20 plants were used for the control treatment and 20 plants were used for the water stress treatment. Four plants per cultivar/treatment for each combination replication were randomly chosen to collect root and shoot dry weight data, six plants per cultivar per treatment were randomly chosen for SOD analysis, and four plants were randomly chosen for leaf water potential measurements. There were six unused plants in each group. Plants were spaced 0.2 m within the row and 0.4 m between rows.

Relative performance measurement. As a mean for comparing the relative performance among cultivars, the following values were calculated for the measured traits.

$$\text{Relative performance (RP)} = \frac{\text{performance under stress}}{\text{performance under control condition}} \times 100$$

Days of water withholding period (DWW) measurement. Before initiating the treatment, all pots were saturated with 500 mL of water per pot in order to create equal moisture conditions in all pots. Water was then withheld until visible wilting symptoms persisted throughout the night in the water stressed plants. Plants were then rewatered and the day of water withholding period (DWW) was recorded for each cultivar. After re-watering the recovery of the cultivars was carefully observed and recorded.

Enzyme extracts, enzyme assay and determination of protein concentration. Our preliminary laboratory studies showed that there were little diurnal changes in SOD activities and based on this observation, leaf samples were collected at 10 AM at 0, 3, 6, and 9 DAT and 2 and 4 DAW. The first fully expanded leaf below the shoot

apex was excised, weighed (≈0.5 g fresh weight), and immediately placed in liquid nitrogen (N₂), lyophilized and stored at –20 °C for SOD activity analysis. The fresh leaf samples (500 mg) were homogenized in 1 mL of 50 mM potassium phosphate and 1 mM EDTA (pH 7.5) buffer. The homogenates were filtered through four layers of cheesecloth and then centrifuged at 4 °C for 15 min at 9400 g. The supernatants were collected and used for SOD assay and protein content after an appropriate dilution.

Leaf superoxide dismutase activity (SOD) was analyzed according to the procedure of Beyer and Fridovich (1987). One enzyme unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of nitro blue tetrazolium (NBT, Wako Pure Chemical Industries, Japan) reduction measured at 560 nm on a spectrophotometer (Hitachi U-2000). The specific activity of SOD was expressed as unit per mg protein, as follows,

$$\text{SOD (unit/mg protein)} = \frac{1/[\text{protein concentration (}\mu\text{g}\cdot\mu\text{L}^{-1}) \times \text{sample}]}{20 \mu\text{L/point of 50\% inhibition rate of NBT} \times 10^{-3}]}$$

Protein content was determined according to Bradford (1976) using bovine serum albumin (BSA) as a standard.

Leaf water potential measurement (Ψ_l). Leaf water potential (Ψ_l) was measured at midday on 0, 3, 6, and 9 DAT and 2 and 4 DAW by the pressure-bomb technique (Tyree and Hammel, 1972), using the uppermost fully expanded leaf (three to four nodes from the shoot apex). Four leaves from each experimental unit were pooled. Leaf stomatal conductances were also measured but the data are not shown here.

Statistical analysis. All data were subjected to analysis of variance (ANOVA) computed by Excel (Microsoft Corp., Redmond, Wash.) and the transformed means were separated by Student's *t* test. Regression analyses were performed by CA-Cricket Graph (Computer Associates, Islandia, N.Y.) program in a Macintosh computer. Regression equations were selected on the best and most significant ($P \leq 0.05$) regression parameters with highest coefficient of determination (r^2).

Results

Dry weight of root, shoot and days of water withholding period effects. Effects of water stress on root and shoot dry weight and days of water withholding period (DWW) are shown in Table 1. The DWW was significantly longer (3 d) in

TM and VF than in RT and KK. Root dry matter significantly decreased by the treatment in all the cultivars. Reduction by water stress was more pronounced in KK and RT than in TM and VF. Similar trends were observed for the effects of water stress on shoot dry weight.

Leaf water potential response (Ψ_l). The effects of water stress on leaf water potential (Ψ_l) are shown in Fig. 1. Ψ_l was decreased by 30% to 40% compared to the untreated control in cultivars tested. The initial reduction was in the range of 20% to 35% and was more rapid in KK and RT than in TM and VF. After re-watering, Ψ_l of stressed plants increased more rapidly in TM and VF than in KK and RT. In KK and RT, Ψ_l in stressed plants was much lower than that of the control at 4 DAW.

Superoxide dismutase (SOD) activity. The effects of water stress on SOD activity are shown in Fig. 2. The SOD activity was increased by water stress in all four cultivars. The increase of SOD activity by water stress was more rapid and pronounced in TM and VF than in KK and RT. After re-watering, SOD activity quickly declined to the control level in all cultivars for stressed plants at 2 DAW except RT, which reached control level at 4 DAW. In the non-stressed control plants, TM and VF had higher SOD activities.

Effects of leaf protein. The effects of water stress on leaf protein concentration of tomato cultivars are shown in Fig. 3. Protein concentration was decreased by the water stress treatments in all the cultivars. A more rapid reduction was observed in KK and RT, than in TM and VF. The KK and RT reached their minimum value of protein content earlier than TM and VF. After re-watering, the protein content increased more rapidly in TM and VF. At 4 DAW, leaf protein contents of stressed plants in TM and VF nearly approached the control levels, but in KK and RT, those of stressed plants remain much lower than those of control plants.

Regression of SOD activities with changes in leaf water potential (Ψ_l). Fig. 4 shows the regression of SOD activities with changes in Ψ_l in each cultivar. The equations were similar for KK and RT and also for TM and VF. Both intercept and slope were significantly different when tolerant cultivars (TM and VF) were compared to sensitive cultivars (KK and RT).

Discussion

As shown in dry matter production, days of water withholding period (Table 1) and changes of leaf water potential (Fig. 1), drought-tolerant cultivars (TM and VF) clearly

Table 1. Effects of water stress on root and shoot dry weight and days of water withholding period.

Cultivars	Root dry wt (g)			Shoot dry wt (g)			DWW ^b
	Control	Stress	RP ^a (%)	Control	Stress	RP (%)	
Kyokko	10.9 a ^c	7.3 b	63	17.9 a	12.9 b	71	10.0 B
Ratan	6.9 a	3.9 b	49	10.9 a	6.8 b	58	9.0 B
TM-0126	12.2 a	9.8 b	79	17.1 a	14.5 b	84	13.2 A
VF134-1-2	10.1 a	7.5 b	71	15.7 a	12.7 b	79	12.7 A

^aRP = relative performance.

^bDWW = days of water withholding period.

^cDifferent letters significantly different at $P < 0.05$. Small letters significantly different between control and stress treatment. Capital letters significantly different among cultivars.

Fig. 1. Effects of water stress on leaf water potential (Ψ_L) in four tomato cultivars. DAT and DAW represent days after treatment and days after re-watering, respectively. Bar indicates standard error ($\pm$ SE). KK = Kyokko, RT = Ratan, TM = TM-0126, and VF = VF134-1-2.

Fig. 2. Effects of water stress on superoxide dismutase (Unit/mg protein) activity of four tomato cultivars. DAT and DAW represent days after treatment and days after rewatering, respectively. Bar indicates standard error ($\pm$ SE). KK = Kyokko, RT = Ratan, TM = TM-0126, and VF = 1134-1-2.

showed improved growth performance under water stress conditions than drought-sensitive cultivars (KK and RT).

Total leaf protein contents were reduced by water stress, but the reduction was more pronounced in drought-sensitive KK and RT than in drought-tolerant TM and VF (Fig. 3). Chen and Tabaeizadeh (1992) demonstrated that water stress promoted syntheses of the stress-specific proteins and repressed the pre-existing protein syntheses in tomato. Although the syntheses of new proteins specific to water stress conditions may have occurred in this experiment, the total protein syntheses are reduced by water stress, probably because of the suppression of energy supply due to reduced photosynthesis and overall adverse effects of stress on biochemical processes. The improved performance of drought-tolerant cultivars under water stress conditions may be related to the ability to minimize the reduction in protein syntheses.

Although total leaf protein syntheses were suppressed by water stress, SOD activities were increased by the stress (Fig. 2). Some of the adverse effects of water stress on plant growth are known to be mediated by the production and accumulation of toxic, reactive oxygen intermediates such as superoxide radicals (Asada and Takahashi, 1987). SOD may act as a scavenging system to protect cells against oxygen radicals that catalyze the reduction of superoxide radicals, and their activities are increased by water stress in many plants (Asada and Takahashi, 1987). In tomato, the increase in SOD activities by water stress were clearly observed in this study and specific cultivar difference were found. In drought-tolerant TM and VF, the increase of SOD activities by water stress was more rapid and pronounced than in drought-sensitive KK and RT. The regression analysis suggests that the slope of the regression equation of drought-tolerant cultivars was much larger than that of drought-sensitive cultivars (Fig. 4), indicating that drought-tolerant cultivars responded to smaller decreases in leaf water potential and increased SOD activities than drought-sensitive cultivars. This suggests that SOD activity is considered to have an important role in drought-tolerant tomato cultivars.

Regression analysis also showed that the intercept of the regression equation was much larger in drought-tolerant cultivars than in drought-sensitive cultivars. This indicates that SOD activities of non-stressed plants were higher in drought-tolerant cultivars than in sensitive cultivars. Our studies suggest that differences in SOD activities may be a useful screening criterion for drought tolerance and it may be possible to select drought-tolerant tomato cultivars on the basis of SOD activities. Further detailed genetic studies on this specific point are necessary.

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Fig. 3. Effects of water stress on leaf protein concentration of four tomato cultivars. DAT and DAW represent days after treatment and days after re-watering, respectively. Bar indicates standard error ($\pm$ SE). KK = Kyokko, RT = Ratan, TM = TM-0126, VF = VF134-1-2.

Fig 4. Correlation between leaf water potential and superoxide dismutase (SOD) activity of tomato under water stress. Asterisks indicate statistical significance at $P < 0.05$. KK = Kyokko, RT = Ratan, TM = TM-0126, and VF = VF134-1-2.