29. Kinetics of Water Deficit-induced Oxidative Stress and the Activation of Antioxidant Enzymes in White Clover Leaves

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화이트 클로버의 수분결핍에 의해 유도된 산화 스트레스의 역동성과 항산화 효소의 활성

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<Objective>

Active oxygen species (AOS), such as peroxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\bullet}OH$), and, in particular, the antioxidative enzymes such as superoxide dismutase (SOD; EC 1.15.1.1), peroxidases (POD; EC 1.11.1.7), catalase (CAT; EC 1.11.1.6) and glutathion reductase (GR; EC 1.6.4.2) that scavenge them have been considered a very important defensive mechanism of plants to a widerange of environmental stresses including extreme temperatures, salinity, water deficit, heavy metals, and pathogen (Elstner, 1982; Smirnoff, 1993; Zhang et al., 1995; Perdomo et al., 1996). Although several works have provided evidence for an effective protector role of POD-CAT-SOD systems against oxidative stress in diverse plant species (Vaidyanathan et al., 2003; Jung, 2004), some physiological aspects remain unclear. In this study, we investigated drought effects on AOS generation, lipid peroxidation and the activities of antioxidant enzymes (SOD, CAT, APOD and GPOD). The physiological relationships among drought-induced activation of antioxidant enzymes, AOS status, and some stress symptomatic parameters in response to the change in leaf-water status were assessed in a kinetic pattern.

<Materials and Methods>

Sods of white clover (*Triforium repens* L.) at full vegetative stage were transplanted to 3 L pot containing a mixture of sand and fritted clay. Control pots were watered every day to maintain constant soil water potential (Ψs) close to -0.04 MPa. Drought stress was imposed by withholding water until Ψs of -0.12 MPa achieved. Each treatment lasted for 28 days and plants tissues were sampled at intervals of 7 days, respectively.

<Results>

Water-deficit stress was imposed during 28 days by decreasing the daily irrigation. Control plants were watered to field capacity. Water-deficient gradually decreased leaf-water status. For the first 7 days, dry mass (DM), H_2O_2 and lipid peroxidation were not significantly affected by water-deficit. From 14 days of treatment, the stress-symptomatic responses were more distinct. Activation of superoxide dismutase (SOD) and catalase (CAT) by water-deficit continued during the early 14 days (leaf water potential, $\Psi_w = -1.65$ MPa) and was correlated with $O_2^{\bullet -}$ and H_2O_2 accumulation. As Ψ_w decreased below -1.65 MPa, activation of guaiacol-peroxidase (GPOD) increased and was significantly correlated with a progressive increase in lipid peroxidation and growth restriction. The data indicate that drought-induced antioxidative enzymes were activated in two distinct phases which were closely correlated with changes in Ψ_w, which in turnwas considered as an easy determinant of stress intensity.

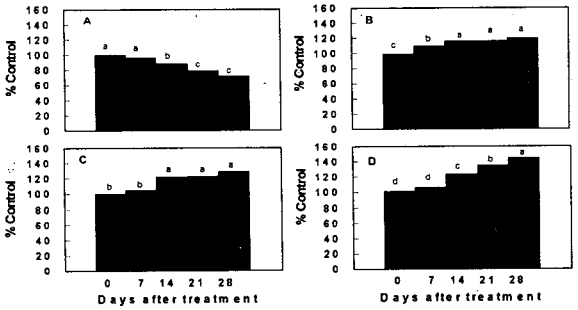

Fig. 1. Water-deficit effects on(A) drymass, (B) $O_2\cdot^-$, (C) H_2O_2 and (D) lipid peroxidation in white clover leaves during the 28 days experimental period. The experimental data obtained in water-deficit stressed leaves were expressed as a percentage ofthose found in the well-watered(control) leaves.

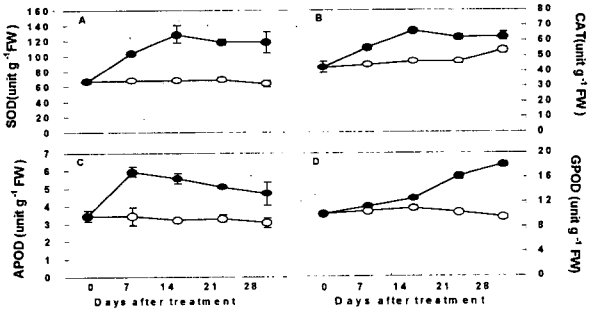

Fig. 2. Changes in the activities of (A) superoxide dismutase, SOD; (B) catalase, CAT; (C) ascorbate-peroxidase, APOD and (D) guaiacol-peroxidase, GPOD in water deficit-stressed and well-watered control leaves of white clover.

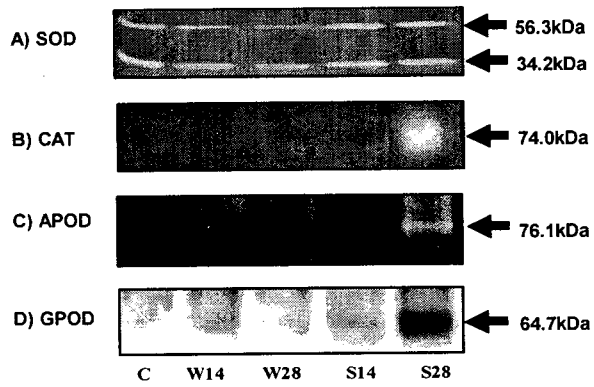

Fig. 3. Activity staining of (A) superoxide dismutase, SOD; (B) catalase, CAT; (C) ascorbate-peroxidase, APOD and (D) guaiacol-peroxidase, GPOD in white clover leaves. Samples were taken at the day of treatment(control, day 0), 14 and 28 days after well-watered (W) or water-deficit (S) treatment.

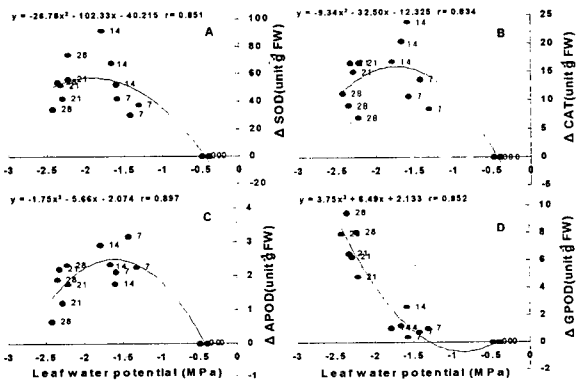

Fig. 4. Relationships between leaf water potential (Ψw) in the water-deficit stressed plants and activity of (A) superoxide dismutase, SOD; (B) catalse, CAT; (C) ascorbate-peroxidase, APOD and (D) guaiacol-peroxidase, GPOD as affected by water-deficit treatment Values are normalized to the difference (Δ) between the enzyme activity measured at the water-stressed leaves and that of the well-watered(control) leaves.

Table 1. Linear relationship between the activity of antioxidant enzymes and $O_2\cdot^-$, H_2O_2, MDA, or DM in two distinct phases of stress development.

	△$O_2\cdot^-$	△H_2O_2	△MDA	△DM
Day 0 ~ Day 14				
△SOD	r = 0.936 ***	r = 0.858 **	r = 0.830 **	r = 0.818 **
△CAT	r = 0.912 ***	r = 0.882 **	r = 0.899 ***	r = 0.642 n.s
△APOD	r = 0.798 **	r = 0.554 n.s	r = 0.500 n.s	r = 0.483 n.s
△GPOD	r = 0.751 *	r = 0.877 **	r = 0.867 **	r = 0.611 n.s
Day 14 ~ Day 28				
△SOD	r = 0.230 n.s	r = 0.228 n.s	r = 0.421 n.s	r = 0.270 n.s
△CAT	r = 0.702 *	r = 0.784 *	r = 0.861 **	r = 0.951***
△APOD	r = 0.331 n.s	r = 0.400 n.s	r = 0.444 n.s	r = 0.365 n.s
△GPOD	r = 0.591 n.s	r = 0.740 *	r = 0.868 **	r = 0.922***