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The Oxidative Stress Induction and Response of Antioxidative Enzymes in the Large Patch-Infected Zoysiagrass

Ⅱ. Activity of antioxidative enzymes

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라지 팻치에 감염된 잔디의 산화적 스트레스 발현과 항산화효소의 활력의 변화

Ⅱ. 항산화효소의 활력

김대현 · 이복례 · 이 명 · 김태환*

요 약

한국형 잔디에 있어 병원성 (라지 팻치) 감염이 산화적 스트레스의 발현에 미치는 영향을 규명하기 위하여 라지 팻치에 감염된 잔디의 항산화 효소의 활성을 조사하였다. 처리 후 6일 동안 이틀간격으로 잎과 뿌리 시료에 대해 각각 분석하였다. 초기 2일 동안 SOD 효소 활성은 처리 간 아무런 차이가 나타나지 않았으나 처리 후 6일차에는 대조구에 비해 라지 팻치에 감염된 잎에서 48% 뿌리에서 49% 각각 높게 나타났다. CAT 효소활성은 처리 후 2일간 잎에서 25% 뿌리에서 101% 각각 증가하였지만 그 이후 급격히 감소하여 4일차에는 대조구에 비해 현저히 낮게 나타났다. 라지 팻치에 감염된 잎에서 POD 활성은 감염 기간 동안 현저히 증가하여 6일 차에는 75% 까지 증가하였다. 뿌리에서 감염에 따른 POD 효소 활성의 증가는 뚜렷하였으며 대조구에 비해 약 2배 높았다. 이러한 결과들은 잔디에 있어 라지 팻치 감염은 산화적 스트레스를 유도하며, 이에 따라 초기 6일간의 병원성 감염에 대한 SOD-CAT-POD 항산화적 기작이 효과적으로 작동됨을 보여준다.

(Key words : Catalase, Peroxidase, Superoxide dismutase, Zoysiagrass)

Ⅰ. INTRODUCTION

Large patch in zoysiagrass is a major disease that affects turf quality and the performance of golf course. One of the important mechanism by which plants are damaged during adverse environmental conditions or pathogen infection is the excess production of reactive oxygen species (ROS), superoxide ($O_2^{-\bullet}$) and hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$). Such oxidative stress has been shown to occur in plants exposed to drought, high temperature, chilling, high light, and

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air pollutants (Smirnoff, 1993; Perdomo et al., 1996; Noctor and Foyer, 1998). Superoxide and H_2O_2 can directly inactivate various macromolecules. Hydroxyl radicals react instantaneously with proteins, lipids, and DNA, causing rapid cell damage. A large increase in ROS is observed upon infection of plants with pathogen or upon elicitor treatment (Inzé and Van Montagu, 1995; Jung et al., 2006). The uncontrolled accumulation of ROS generates an interacellular stress and may cause membrane rigidification, peroxidation of membrane lipid, protein denaturation, DNA mutation, and oxidation of macromolecules (Smirnoff, 1993; Menconi et al., 1995). Therefore, detoxification of active oxygen is essential to protect plant tissue against its detrimental effects. It has been well documented that plants are endowed with an array of antioxidant enzymes to cope with ROS. Plant have evolved non-enzymatic and enzymatic protection mechanisms that efficiently scavenge ROS. Antioxidants, such as ascorbic acid (vitamin C), glutathione, α-tocopherols, and carotenoids, occur in high concentrations in plants.

Hydroxyl radicals are too reactive to be eliminated enzymatically, but formation is limited by scavenging of $O_2^{-\bullet}$ and H_2O_2. Superoxide dismutase (SOD; EC 1.15.1.1) can convert the O_2^- free radical into H_2O_2 and O_2. The activity of SOD increased in cool-season grasses during most periods of soil surface drying (Fu and Huang, 2001). Catalase (CAT; EC 1.11.1.6) breaks down H_2O_2 into H_2O and O_2. The decrease in CAT activity in response to low temperature has been reported in cucumber seedling (Omran, 1980). Peroxidase (POD; EC 1.11.1.7) is an oxido-reductive enzyme that participates in the cell wall lignification processes such as oxidation of phenols, suberization, and lignification of host plant cells during the defense reaction against pathogenic agents (Jung et al., 2004). The induction of peroxidase has been associated with disease resistance in a number of plant-pathogen interaction. Other enzymes involved in the oxidation-reduction cycle are monodehydroascorbate reductase, dehydroascorbate reductase, and glutathione reductase. Recently, several cDNA clones encoding glutathione peroxidases have been isolated, suggesting that in plants, as in animals, these proteins might play an important role in scavenging H_2O_2 and/or in alleviating lipid peroxidation.

We hypothesized that zoysiagrass adaptation to large patch infection could be associated with the maintenance or increase in the activity of antioxidant enzymes. This experiment was designed to examine this hypothesis by comparing activities of enzymatic antioxidants, including SOD, CAT, and POD between healthy (control) and pathogen-infected zoysiagrass plants.

Ⅱ. MATERIALS AND METHOD

1. Plant culture and experiment procedure

Sods of zoysiagrass (*Zoysia japonica*) were taken from healthy fairway for control or from the sites where the symptom of large patch infection has already appeared for pathogen-infected treatment at Muan CC, Chonnam, Korea. Unless otherwise stated, plant culture, treatment, and experiment procedure are same with the methods previously described (Kim et al., 2007).

2. Measurement of antioxidant enzyme activities

For extraction of enzymes, fresh samples (0.5 g) were homogenized with 1.5 mL of 100 mM K-PO_4 buffer solution (pH 7.0) containing 2 mM phenylmethylsulfonyl fluoride (PMSF), and centrifuged at 14,000 g at 4℃ for 20 min. Protein concentration was determined using the method

of Bradford (1976).

The activity of superoxide dismutase (SOD) was determined by measuring its ability to inhibit the photoreduction of nitroblue tetrazolium (NBT) following the method of Giannopolitis and Ries (1977). One unit of enzyme activity was defined as the amount of enzyme required to inhibit 50% of the NBT photoreduction in comparison with tubes lacking the plant extract. Catalase (CAT) activity was assayed using the method of Mishra et al. (1993). The reaction mixture of 1 mL contained 0.5 mL of 100 mM potassium phosphate buffer (pH 7.0), 0.1 mL of 110 mM H_2O_2 and enzyme extract. The decrease in absorbance at 240 nm was recorded as a result of H_2O_2 degradation (extinction coefficient of 36 mM^{-1} cm^{-1}). For peroxidase (POD) activity, the oxidation of guaiacol was estimated by measuring the increase in absorbance at 470 nm for 1 min (Lee and Lin, 1995). The reaction mixture contained 50 μL of 20 mM guaiacol, 2.8 mL of 10 mM phosphate buffer (pH 7.0), and 50 μL of enzyme extract. Reaction was initiated by adding H_2O_2 and the activity was calculated using an absorption for tetraguaiacol (26.6 mM^{-1} cm^{-1}). One unit of enzyme activity was defined as the amount of enzyme that causes the formation of 1 μM tetraguaiacol per min.

III. RESULTS

1. SOD activity

The changes in SOD activity during 6 days of measurement are presented in Fig 1. In leaves, the significant increase in SOD activity, caused by pathogen infection, occurred from day 2. The increase continued and reached at 48% higher level at day 6 compared with the control. At day 0, SOD activity in root of infected plants was already higher than that of control, and then continuously increased by 49% compared to the control at day 6. However, no significant changes occurred in control plants.

2. CAT activity

The changes in CAT activity in leaves and roots of pathogen-infected or control plants are presented at Fig. 2. The CAT activity of

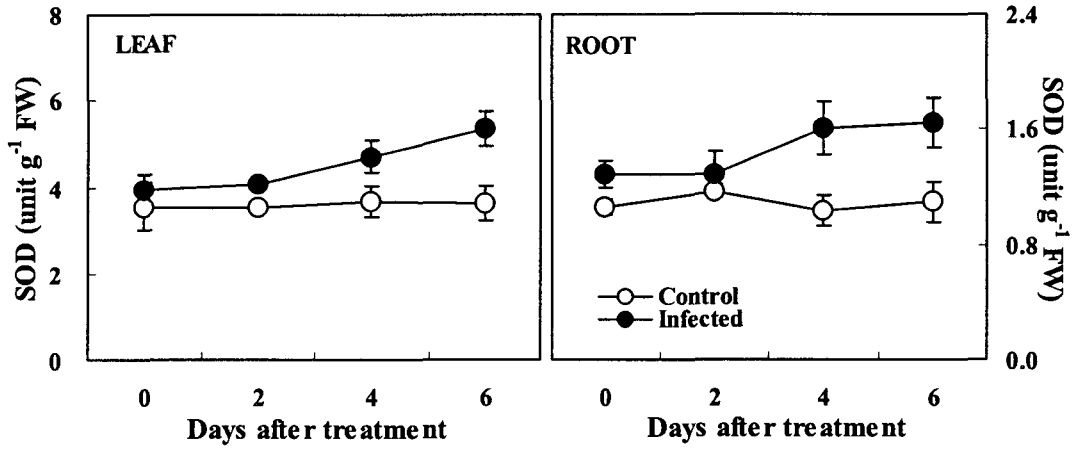

Fig. 1. Changes of SOD activity in leaves and roots of the pathogen-infected or healthy (control) zoysiagrass during 6 days of measurement. Each value is the mean ± S.E. for n=3.

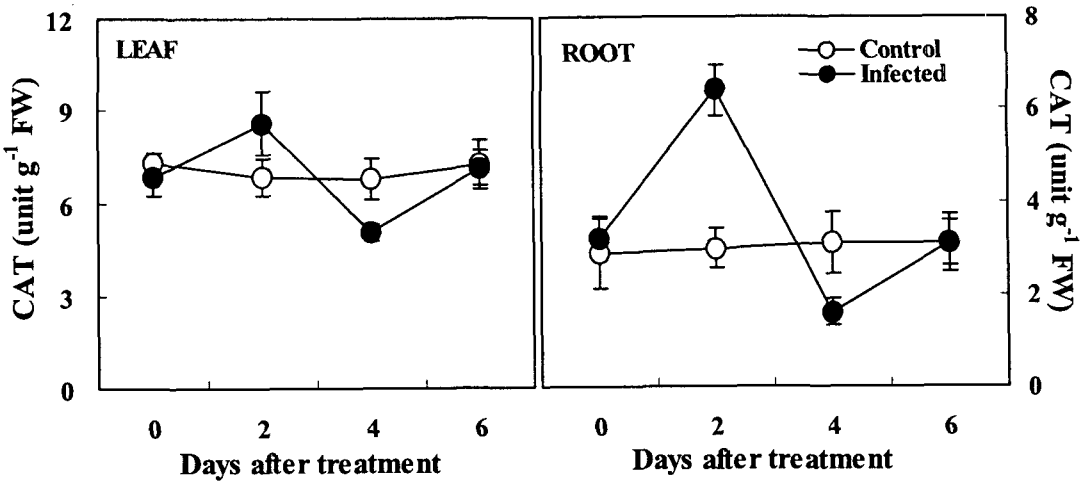

Fig. 2. Changes of CAT activity in leaves and roots of the pathogen-infected or healthy (control) zoysiagrass during 6 days of measurement. Each value is the mean ± S.E. for n=3.

pathogen-infected plant rapidly increased in leaf (+25%) and root (+101%) within 2 days of treatment, while that of the control plants was less varied. From day 2, however, CAT activity was significantly decreased in both of leaf (- 26 %) and root (- 47.7%) compared to control, and then it was recovered to the control level.

3. POD activity

The changes in POD activity in control and pathogen-infected plants during 6 days of measurement are presented at Fig. 3. POD activity in the control was less changed within the range of 5.92~6.21 unit g^{-1} FW for leaves

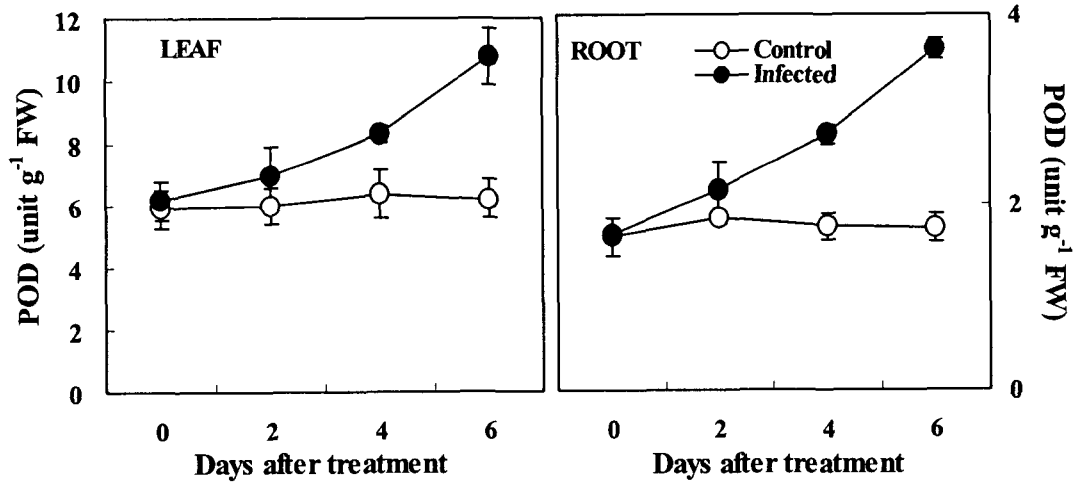

Fig. 3. Changes of POD activity in leaves and roots of the pathogen-infected or healthy (control) zoysiagrass during 6 days of measurement. Each value is the mean ± S.E. for n=3.

and 1.64~1.74 unit g^{-1} FW for roots (Fig. 3). The POD activity in pathogen-infected leaves continuously increased by 74% throughout 6 days of treatment. In the pathogen-infected roots, POD activity also continuously increased throughout the experimental period, showing 2-fold higher activity compared with the control at day 6.

Ⅳ. DISCUSSION

One of the earliest plant responses to pathogen infection is the accumulation of reactive oxygen species (ROS), such as O_2^- and H_2O_2 at the infection site (Lamb and Dixon, 1997). The reactive nature of AOS makes them potentially harmful to all cellular components. Fortunately, plants have the capacity to cope with these reactive oxygen species by eliminating them with an efficient AOS-scavenging system (Slooten et al., 1998). Higher scavenging activity may correlate with enhanced abiotic stress tolerance of the plants (Bowler et al., 1992).

In the present work, a significant increase of SOD activity was observed in pathogen-infected plants after 2 days. Much higher induction of SOD activity might be associated with it having an effective role in protection against pathogen infection stress. Vanacker et al. (1998) suggested that an increase in SOD activity following pathogen attack might be required to catalyze the synthesis of H_2O_2 during the oxidative burst and to prevent accumulation superoxide. Similarly, an increase in SOD activity was observed in potato / *Phytophthora infestans* (Doke et al., 1983), tobacco / *Peronospora tabacina* (Ederva et al., 1991), coffee / *Hemileia vastatrix* (Daza et al., 1993) and pearl millet/ *Sclerospora graminicola* (Babitha, 2002).

In contrast to SOD, CAT activity was dramatically increased in pathogen-infected plants during the first 2 days, compared with the control. This suggests that the CAT is thought to play an important role in removing H_2O_2 from plant tissues in early stage (Wu et al., 1997). A similar results reported by Garcia-Limones et al. (2002) showed that CAT and SOD activities are early enhanced in the incompatible interaction between chickpea and *Fusarium oxysporum f. sp. ciceris*. Significant induction of CAT activities has also been observed in the compatible interaction between H. *vulgare* and B. *graminis* Alg-S (Vanacker et al., 1998). However, CAT activity was suppressed later in pathogen-infected plants. This suppression may result in the accumulation of H_2O_2, which can react with O_2^- to produce hydroxyl free radicals via the Herbert-Weiss reaction (Elstner, 1982). Suppression of CAT activity has also been observed in incompatible plant-pathogen interactions (Ádám et al., 1995; Milosevic and Slusarenko, 1996). These results indicated that the ability of CAT to scavenge AOS was activated during initial and was limited during severe cell damage by pathogen infection.

We have also observed in this work that POD activity was significantly increased by pathogen infection after 2 days of treatment. Similar results reported by Silva (2004) that the high POD activity in pathogen-infected tomato plants were usually found at a later stage of infection. Enhanced peroxidase activity very often is associated with resistance phenomena such as phenylalanie ammonia lyase activity (Rahtmell, 1973; Tena and Valbuena, 1983). This result suggested that the increased POD activity for the later period might have been involved in process affecting polysaccharides in the cell wall such as oxidation of phenols and lignification which results in growth restriction, contributing

to plant cell wall reinforcement during fungal penetration, and might also be involve in scavenging H_2O_2 (lamb and Dixon, 1997; Mittler, 2002).

Taken together, the data obtained from this study indicated that SOD-CAT-POD antioxidant system of zoysiagrass was effectively operated in scavenging superoxide and peroxide radicals generated by pathogen infection-induced oxidative stress.

V. ABSTRACT

To investigate the effect of large patch infection on oxidative stress induction, superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) were compared between pathogen-infected and healthy (control) zoysiagrass. The sampling for leaves and roots were carried out every 2 days for a period of 6 days. The SOD activity was not significantly affected by pathogen-infection until day 2, but significant increase of both leaves (+48%) and roots (+49%) were observed at day 6 compared with control. The CAT activity was remarkedly increased by +25% in leaves and +101% in roots within the first 2 days and then rapidly decreased. The POD activity in pathogen-infected leaves was significantly increased by 74% at day 6. The increase of POD activity in pathogen-infected roots was 2-fold higher than that of the control at day 6. These results indicated that large patch-infection induce oxidative stress, and that SOD-CAT-POD antioxidant system of zoysiagrass was effectively operated.

VI. REFERENCES

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