韓草誌 (J. Korean Grassl. Sci.) 27(1) : 37~44, 2007

Proline and Ammonia Accumulation in the Zoysiagrass Infected with Large Patch

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라지 팻치에 감염된 잔디에서 프롤린과 암모니아의 축적

김대현 · 이복례 · 이재식 · 이 명 · 김태환

요 약

병원균 감염에 의한 식물체내 프롤린과 암모니아의 농도 변화와 그것의 스트레스 생리학적 의미를 구명하기 위하여 *Rhizoctonia spp.*를 처리 후 라지 팻치에 감염된 잔디의 생육 및 관련 화학적 성분을 감염이 되지 않은 대조구와 비교하였다. 라지 팻치에 감염된 잔디의 뿌리의 건사율은 대조구에 비해 약 30% 증가 하였다. 가용성 단백질 농도는 병원균 처리후 6일째 잎의 경우를 제외하고는 라지 팻치 감염에 따른 유의적인 영향이 없었다. 암모니아 농도 역시 라지 팻치에 감염된 잎과 줄기에서 공히 유의적으로 증가하였다. 프롤린 농도는 잎과 뿌리에서 대조구에 비해 각각 3.4 및 4.5배 증가하였다. 이러한 결과들은 잔디에 있어 병원균 감염에 따른 프롤린의 축적은 스트레스 강도를 나타내는 민감한 표지물질로서 의미가 있음을 제시한다.

(**Key words** : Ammonia, Large patch, Proline, Zoysiagrass)

I. INTRODUCTION

Disease in turfgrass is a main stress factor along with abiotic stresses such as high temperature, freezing, salinity, and drought. Turf diseases caused by *Rhizoctonia spp.* are reported in many researches (Martin and Lucas, 1984; Oniki, et al., 1986; Burppee, 1980). Large patch in zoysiagrass is a major disease that affects the performance on the golf course. This disease declines turf quality by stress in plant growth. During prolonged period of stress, the decrease in water availability for transport-associated processes leads to changes in the concentrations of many metabolites, followed by disturbances in amino acid and carbohydrate metabolism. Several classes of compounds have been observed to increase the synthesis of compatible solutes such as special amino acids (e.g. proline), sugars and sugars-alcohols, and glycine-betaine (Yancey et al., 1982; Girousse et al., 1996). A large number of plant species accumulate proline in response to abiotic stresses (Aspinall and Paleg, 1981; Delauney and Verma, 1993). Therefore, it has been suggested that proline is involved in stress tolerance by playing a role in counteracting

"이 논문은 2005년도 전남대학교 학술연구비 지원에 의하여 연구되었음(This study was financially supported by Chonnam National University)"

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the effects of various stresses (Delauney and Verma, 1993; Hare et al., 1998). Stress-mediated changes in proline biosynthesis, including hydrolysis of proteins and oxidative degradation processes, increased the proline level of plants exposed to different stresses (Rayapati and Stewart, 1991). The degradation of proline was almost completely inhibited in stressed plants (Rayapati and Stewart, 1991). However, the rate of proline oxidation in non-stressed plants was too low to explain the measured high rates of proline accumulation under stress. Therefore, the increase in proline content in stressed plants is predominantly associated with the *de novo* synthesis (Gzik, 1996; Kim et al., 2004). Moreover, abiotic stresses that reduce leaf or shoot growth increase production of ammonia, leading to its removal through *de novo* synthesis of arginine (Rabe and Lovatt, 1986; Lovatt, 1990), which is a precursor of proline. Proline accumulation has been proposed as a mechanism for storage of excess N (Stewart and Boggess, 1977). Proposed roles in stress tolerance of proline include serving as osmotica, stabilizing macromolecules and membranes, and detoxifying tissues of excess N (Rabe, 1990; Huang et al., 1994). Despite the extensive researches to elucidate the roles of proline in ameliorating the detrimental effects of plant stresses, the physiological significance of its accumulation under stressed condition induced by pathogen-infection remains equivocal. This has led to the hypothesis that pathogen-infection may influence on protein synthesis and the concentration of nitrogen containing compounds symptomatic of plant stresses. This experiment was designed to approve this hypothesis by comparing proline, ammonia, and protein concentration between healthy (control) and large patch infected zoysiagrass. Correlations between root mortality and physiological parameters were also assessed.

II. MATERIALS AND METHODS

1. Plant culture and experimental procedure

Sods of zoysiagrass (*Zoysia japonica*) were taken from healthy fairway for control or from the sites where large patch disease appeared for pathogen-infected treatment at Muan CC, Chonnam, Korea. They were transplanted to 3 L pot containing a mixture of sand and fritted clay. During 2 weeks of adaptation, 10 mL (average of 1×10^5 zoospores per milliliter) of *Rhizoctonia spp.* was inoculated every 3 days as a pathogen inoculum. Sampling for each treatment was begun from the end of adaptation (day 0), and continued every 2 days for a period of 6 days. Harvested plants were separated into leaves + stolon and roots. Tissue samples were immediately frozen in liquid nitrogen. Freeze-dried samples were finely ground and stored under vacuum for further analysis.

2. Root mortality

Root mortality was measured using the modified method of Knievel (1973). The fresh roots (500 mg) were incubated with 10 mL of 0.6% 2,3,5-triphenyltetrazolium chloride in 0.05 M phosphate buffer, pH 7.4, for 24 h in the dark at 30℃. Roots were then rinsed twice with deionized water. Formazan was extracted twice from roots with 95% ethanol at 70℃ for 4 h. Combined extracts were adjusted to a final volume of 50 mL with 95% ethanol. Absorbance was read at 490 nm. A standard curve was made using different proportions of living roots

and killed roots to calculate root mortality. Root mortality was expressed as percentage dead root dry weight (DW) of the total root DW.

3. Chemical analysis

Soluble proteins were extracted by suspending freeze-dried sample with 100 mM sodium phosphate buffer (pH 7.0). Tubes were vortexed for 30 sec 4 times and centrifuged 13,000 g for 10 min, and the supernatant was retained. The pellet was re-extracted. Soluble proteins in the combined supernatant were quantified using dye-binding method (Bradford, 1976).

For ammonia determination, 2 g of fresh tissue was immediately homogenized with 10 mL of 10% (w/v) trichloroacetic acid (TCA) and then centrifuged at 10,000 g for 10 min at 4°C. After microdiffusion of the acid soluble supernatant fraction containing the combined pool of NH_3-NH_4^+ as NH_4^+, in Conway dishes, colorimetric determination of NH_4^+ was performed with Nessler's ammonium color reagent on an aliquot of solution (Kim and Kim, 1996).

Proline was extracted and its concentration was determined by the method of Bates et al. (1973). Leaf segments were homogenized with 3% sulfosalicylic acid and the homogenate was centrifuged at 3,000 g for 20 min. The supernatant was treated with acetic acid and acid ninhydrin, boiled for 1 h and then the absorbance was determined at 520 nm with a UV-1601 Shimadzu spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Proline concentration was calculated with L-proline as the standard.

III. RESULTS

1. Root mortality

The changes in root mortality during 6 days are presented in Fig. 1. At day 0, the root mortality of infected plant was already significantly higher than that of control, and then continuously increased to 62% at day 6. However, no significant change was occurred in non-infected (control) plants.

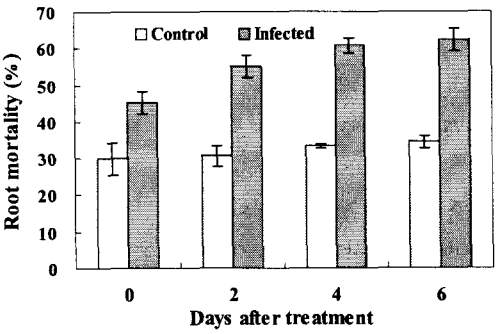

Fig. 1. Root mortality in pathogen-infected or healthy (control) zoysiagrass during 6 days of measurement. Vertical bars indicate the standard error (SE) for n=3.

2. Soluble protein

The changes in soluble protein concentration in leaves and roots of pathogen-infected or control plants are presented in Fig. 2. In control plants, the mean concentration was nearly constant within a range of 1374 - 1413 $\mu g\ g^{-1}$ FW for leaves and 250 - 273 $\mu g\ g^{-1}$ FW for roots, respectively, during 6 days of the measurement. In pathogen-infected plants, the concentration of soluble protein in leaves decreased by about 12% at day 6 compared to the initial level, whereas no significant changes were observed in roots.

3. Ammonia and proline concentration, and their relations with root mortality

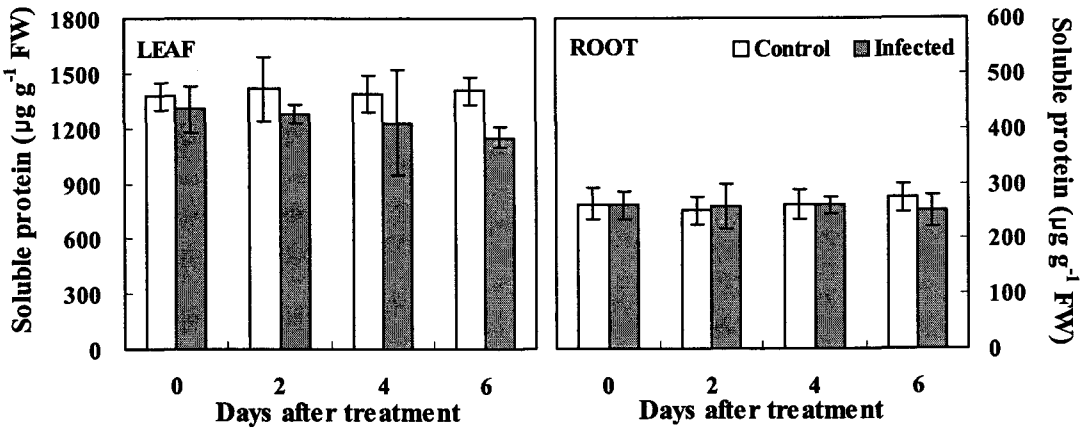

Fig. 2. Changes of soluble protein in leaves and roots of pathogen-infected or healthy (control) zoysiagrass during 6 days of measurement. Vertical bars indicate the standard error (SE) for n=3.

The effects of pathogen-infection on ammonia and proline concentrations are presented in Fig. 3 and Fig. 4, respectively. Ammonia concentration in control was less changed within the range of 26.7 - 32.2 for leaves and 16.8 - 18.2 μmol g^{-1} DW for roots compared with those of the pathogen-infected plants (Fig. 3). A gradual increase by pathogen-infection was observed in both leaves and roots. At day 6, the significant ($p \leq 0.05$) increases of ammonia in leaves (+27.6%) and in roots (+39.7%) of pathogen-infected plants were marked. Proline concentration in both leaves and roots of healthy (control) plants was not changed significantly throughout the experimental period (Fig. 4). Pathogen-infection continuously increased proline concentration in both leaf and root tissues. Proline concentrations in the pathogen-infected plants at day 6, the highest level, were 3.4- and 4.5-fold higher than those of leaves and roots of

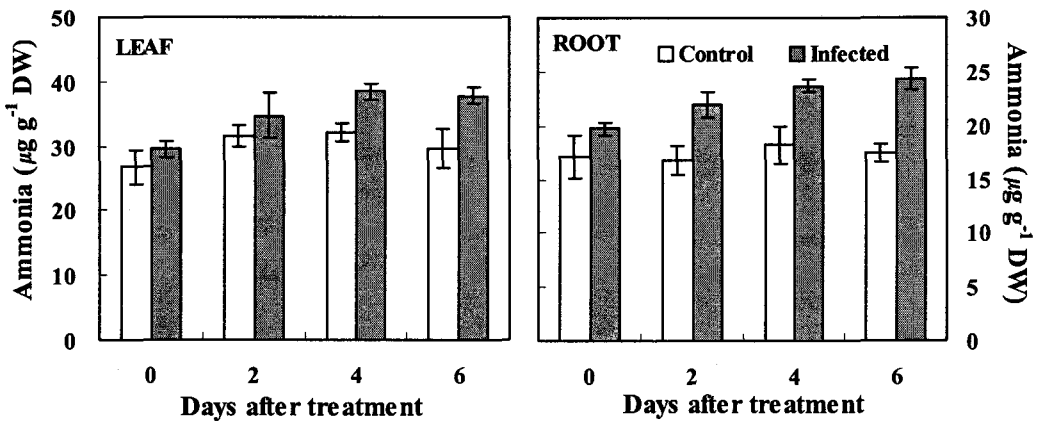

Fig. 3. Changes of ammonia in leaves and roots of the pathogen-infected or healthy (control) zoysiagrass during 6 days of measurement. Vertical bars indicate the standard error (SE) for n=3.

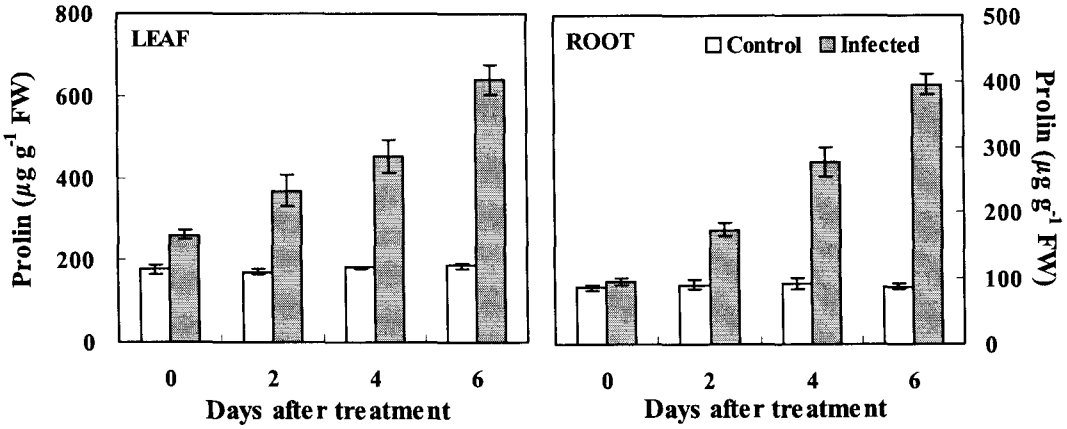

Fig. 4. Changes of proline in leaves and roots of the pathogen-infected or healthy (control) zoysiagrass during 6 days of measurement. Vertical bars indicate the standard error (SE) for n=3.

control, respectively.

To examine ammonia and proline responses to the stress intensity induced by pathogen-infection, the linear relationships with roots mortality were assessed in pathogen-infected and control plants (Table 1). The concentration of soluble protein was not related significantly with root mortality under both treatments. Under pathogen-infected condition, the increase in ammonia concentration was highly ($p \leq 0.001$) related with root mortality. The positive relationship between the increase in root mortality and proline accumulation was found in leaves ($r= 0.769$, $p \leq 0.01$) and in roots ($r=0.876$, $p \leq 0.001$) of pathogen-infected plants.

IV. DISCUSSION

Increased root mortality occurred prior to decline in turf quality and shoot growth in response to pathogen-infection. Decline of root activities could adversely affect shoot growth by

Table 1. Linear correlation between root mortality and some physiological parameters (soluble protein, ammonia and proline) in control or pathogen-infected plants

		Soluble protein ($\mu g\ g^{-1}$ FW)	Ammonia ($\mu mol\ g^{-1}$ DW)	Proline ($\mu g\ g^{-1}$ FW)
Root mortality (%)				
Leaves	Control	$r = 0.046^{n.s}$	$r = 0.197^{n.s}$	r = 0.566*
	Pathogen-infected	$r = 0.346^{n.s}$	r = 0.851***	r = 0.769**
Roots	Control	$r = 0.120^{n.s}$	r = 0.713**	$r = 0.446^{n.s}$
	Pathogen-infected	$r = 0.051^{n.s}$	r = 0.898***	r = 0.876***

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; n.s, non-significant

limiting nutrient supply from roots to shoots (Kramer, 1983). During the experimental period, root mortality in pathogen-infected plants was increased about 2-fold at day 6 (Fig. 1) and stress-related detrimental symptoms caused by pathogen-infection appeared in zoysiagrass. Although pathogen-infection retarded plant vegetative growth, they could survive by acquired stress resistance by pathogen. In this study, root mortality was increased highly in pathogen-infected plants, but not exceeded 60% at the end of experiment (day 6). This result suggests that resistance mechanisms are possibly being in the process in response to pathogen-infection. In this experiment, pathogen-infection decreased soluble protein concentration by 12%, but not significantly changed in roots (Fig. 2). Studies on the effect of stress on protein concentration in plant showed variable results, which might be due to the difference in tissue type and stress severity. However, a reduction in soluble proteins is a common observation (Moran et al., 1994; Gilbert et al., 1998; Kim et al., 2004) that is usually explained by a reduction in N availability and the limitation of both N acquisition and nitrate reductase activity under stressed conditions (Aslam et al., 1984; Rao and Gnanam, 1990; Serraj et al., 1999). Although certain stresses lead to a decrease of N content in plant tissues, the accumulation of nitrogen-containing compounds (NCCs) under stressed condition has been widely documented (Dubay and Pessarakli, 1995; Rabe, 1999; Serraj et al., 1999). As expected, pathogen-infection significantly increased ammonia concentrations in both leaves and roots (Fig. 3). Recently, in white clover the ammonia accumulation has been found to closely related with the inhibition of *de novo* protein synthesis, which was caused by drought stress (Kim et al., 2004) and with an transient increase of *de novo* amino acid synthesis as a transient adaptive response (Lee et al., 2005). Furthermore, a highly significant relationship between the increase in ammonia concentration and proline accumulation was observed under drought-stressed condition (Kim et al., 2004), where in turn *de novo* protein synthesis was inhibited. In this study, pathogen-infection increased the proline concentration in both leaf and root tissues. Proline accumulation in response to stress (Aspinall and Paleg, 1981; Delauney and Verma, 1993) and a positive relationship between this amimo acid and the level of stress tolerance (Delanney and Verma, 1993; Hare et al., 1998) has been documented in a large number of plant species. The function of proline is often associated with osmotic adjustment, by lowering the water potential to improve the uptake of water against the external gradient, but a number of other roles for this compound have been hypothesized in recent literature (Rabe and Lovatt, 1984; Vernon et al., 1993; Gilbert et al., 1998). Possible roles include: serving as a readily available energy or nitrogen source during limited photosynthesis and growth, detoxification of excess ammonia under periods of stress (Huang et al., 1994; Rabe, 1999), and stabilization of enzymes and/or membranes (Sivakumar et al., 1998).

The present data showed that the increases in ammonia and proline concentration were highly related with root mortality in pathogen-infected plants (Table 1). However, their relationships in healthy (control) plants were not significant or much lower. These results suggest that proline accumulation is one of the stress-responsive symptoms related to excess of ammonia production, which was possibly attributed to the

decrease in *de novo* protein synthesis in pathogen-infected zoysiagrass, and this might be a useful indicator for plant stress intensity caused by pathogen-infection.

V. ABSTRACT

To investigate the response of proline and ammonia to pathogen infection, plant growth and relevant chemical component were examined in large patch-infected or healthy (control) zoysiagrass during 6 days after treatment. Pathogen-infection increased root mortality by 30% compared to control. Soluble protein was not significantly affected by pathogen-infection except in the leaf at day 6. Ammonia concentration also increased significantly in both leaves and roots of pathogen-infected plants. Proline concentration in leaves and roots increased to 3.4- and 4.5-fold, respectively, compared to those of control at day 6. These results suggest that proline accumulation may be a sensitive biochemical indicator representing the stress intensity caused by pathogen infection in zoysiagrass.

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