Inter-ramet Physiological Integration Detected in Buffalograss (*Buchloe dactyloides* (Nutt.) Engelm.) under Water Stress

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수분스트레스 하에 있는 버팔로그래스에서 검출된 무성생식체의 생리학적 조정

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ABSTRACT

Buffalograss is an important turfgrass species with excellent cold, heat, and drought tolerance. Understanding the physiological integration of buffalograss under heterogeneous conditions helps to develop cultural practices that better use limited resources for uniform turf quality. The objective of this study was to evaluate physiological integration of buffalograss under water deficit stress and the involvement of lipid peroxidation and antioxidants in the process. In one experiment, buffalograss was planted in the center of a four-compartment growth unit. Watering frequencies, once a week(+) and once in two weeks(-), were combined with the sand(S) or peat(P)

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in each unit to generate five total treatments(P+S-P-S+, P+P+P+P+, S-S-S-S-, P-P-P-P-, and S+S+S+S+). The average number of shoot established from the heterogeneous root-zone medium was higher than the average of four possible homogeneous media. In second experiment, single ramet in Hoagland solution(S_0) or single ramet in Hoagland solution with 20% PEG-6000(S_s) were compared with two connectedramets under different treatments. Treatments for connected ramets were young ramet in Hoagland solution(Y_{os}) and old ramet in Hoagland solution with 20% PEG-6000(O_{os}), and old ramet in Hoagland solution(O_{ys}) and young ramet in Hoagland solution with 20% PEG-6000(Y_{ys}). Lipid peroxidation, antioxidants, and proline showedphysiological integration between ramets subjected to different levels of water stress. Superoxide dismutase(SOD), Guaiacol peroxidase(G-POD), malondialdehyde(MDA), and free proline also showed different time courses and relative activities during the physiological integration.

Key words: Turfgrass management, ecology, lipid peroxidation, antioxidant

INTRODUCTION

Physiological integration is an important means by which clonal plants adapt to heterogeneous distributions of available resources(Pitelka and Ashmun, 1985; Marshall, 1990). Assimilated resources in clonal plants are redistributed among the interconnected ramets according to source-sink relationships(Kaitaniemi and Honkanen, 1996). Previous research on clonal species have shown that ramets under stress may be subsidized by the unstressed ramets(Hutchings, 1999). Under heterogeneous conditions, clonal plants often are able to produce more biomass than separated ramets under homogeneous conditions when the amount of resource is limited(Marshall, 1990; Hellström et al., 2006). Resource sharing in clonal plants is well documented and is sometimes reflected in the morphological plasticity duringphysiological integration. For instance, differentiation of a clonal plant into parts of labor and foraging functions may be enhanced when essential nutrients and light are heterogeneous(Stuefer et al., 1994; Sergio et al., 2007). It is assumed that an internal gradient will establish when connected ramets are exposed to different resource availability(Marshall, 1990), and such gradient may serve as a driving force for redistribution subject to vascular constraints(Marshall and Anderson-Taylor, 1992). In connected ramets of *Fragaria chiloensis* water transportation from parent ramets to daughter plants along the water potential gradient was reported(Alpert and

Mooney, 1986). In rhizomatous carex species, water movement from ramets with sufficient water supply to ramets under drought stress was also noticed(Kroon, et al., 1996). However, all transportation of resources does not follow concentration gradients in clonal species(Alpert et al., 2002). The mechanisms of physiological integration without concentration gradient are not clear.

Buffalograss is a typical stoloniferous clonal species with excellent cold, heat, and drought tolerance. It is widely used in low maintenance areas such as cemeteries and parks, and is increasingly used in golf courses and other sports fields. Spatial and temporal variations in soil conditions are often causes of low turf qualities such as localized dry spots(Brian, 2002), and infestations of weeds, diseases, and insects (Potter, 2005). Many turfgrass management practices are designed to correct such problems, such as aeration, topdressing, and application of wetting agents. All stoloniferous and rhizomatous grass species are not clonal integrative(Schmid and Bazzaz, 1987; Price and Hutching, 1992). Understanding the physiological integration of buffalograss helps to develop cultural practices that better use limited resources for uniform turf quality.

Biochemical responses in turfgrass under stress have been intensively researched. For instance, changes in enzymes and hormones(Zhang and Schmidt, 1999; Liu and Huang, 2002) and the balance of lipid peroxidation and antioxidative enzymes are considered important in the regulation of turfgrass growth and development(Xu and Huang, 2004). However, little is known about the roles of antioxidants in the physiological integration in buffalograss. The objective of this study was to evaluate physiological integration of buffalograss under drought stress and the involvement of lipid peroxidation and antioxidants in the process.

Materialsand Methods

All plant materials used in this study were a clone from a vegetative propagule of buffalograss 'Texoka' after ten vegetative regenerations in a greenhouse. While still connected to the parent plants, new ramets were individually rooted in plastic pots (10×10×10cm) containing sieved and acid-washed sand(<2mm) and peat in 1:1 volumetric ratio. The stolons were cut into segments before experiment with either an individual ramet or a pair of connected ramets of uniform sizes with five to six leaves.

The roots of every ramet were cultured in a 5-L plastic flume filled with Hoagland solution(Ψ_w~ -0.05 MPa) for a week before assigned to different experiments in a greenhouse under 32/25℃(day/night). The hydroponic solution was aerated periodically with an air pump.

The first experiment was initiated on 13 May, 2007. Five different root zone conditions were created in field plots consisting of soil types and watering regimes. Each treatment unit consisted of four square compartments(60×60×50 cm) arranged in a rectangular shape. The inside of each compartment was lined with plastic films to prevent water and nutrient exchanges between the neighbor frames. For heterogeneous conditions, two compartments in each unit were filled with peat(P) (Maihe Kou, Liaoning, China), and the other two were filled with sand(S) (sieved and acid washed) thus creating a heterogeneous root-zone material distribution. In the homogenous treatments, all four compartments were filled with either sand or peat only. Watering frequencies, once a week(+) and once in two weeks(-) were combined with the soil types in each unit to generate five total treatments(P+S-P-S+, P+P+P+P+, S-S-S-S-, P-P-P-P-, and S+S+S+S+). In the center of each of the four-compartment unitwas positioned a parent plant. All treatments were replicated four times in a randomized complete block design. The number of shoots in each and every compartment was recorded every two weeks until the end of the study.

In the second experiment, single ramets and ramet pairs prepared as above were removed from Hoagland solution after pre-culture and treated with different concentrations of polyethylene glycol 6000(PEG 6000) to simulate drought stresses. The single ramets subjected to Hoagland solution(pH=5.8~6.0) with 0%(Ψ_w ~ -0.05 MPa) and 20% PEG-6000(Ψ_w ~ -0.75 MPa) were denoted as S_0 and S_s, respectively. For paired ramets, young ramet in Hoagland solution(denoted as Y_{os}) and old ramet cultured in Hoagland solution with 20% PEG-6000 (pH=5.8~6.0) (denoted as O_{os}) was tested as old ramet under stress. Similarly, old ramet in Hoagland solution(denoted as O_{ys}) and young ramet cultured in Hoagland solution with 20% PEG-6000 (pH=5.8~6.0) (denoted as Y_{ys}) was tested as young ramet under stress. The experiment was in a randomized complete block design with three replications and about 300 ramets in each treatment unit.

Leaf blades of the mature leaves were collected at 12, 24, 36, 48, 60, and 72 h after the initiation of the treatments. After weighing, the samples were wrapped with aluminum foils separately and frozen in liquid nitrogen immediately for 5min and then stored at -80°C for further biochemical analysis.

About 0.2-0.5g of frozen leaf samples were ground in liquid nitrogen and homogenized in 6ml 50mM potassium phosphate buffer(pH 7.8) containing 1mM EDTA and 1gL^{-1} polyvinylpyrrolidone-40(PVP-40) following the method used by Jiang and Zhang(2002). The homogenate was centrifuged at 12000×g for 20 min at 4°C and the resulting supernatant was used for the assays of SOD, G-POD.

Total SOD activity was measured according to the method by Giannopolitis and

Ries(1977). One-hundred microliter of enzyme supernatant was added to 3-ml reaction mixture in a 50mM potassium phosphate buffer(pH 7.8) system containing 2.7mL of 13mM methionine(dissolved with redistill water), 0.1ml of 75μM ρ-nitro blue tetrazolium chloride(NBT), 0.1ml of 10μM EDTA and 0.1ml 2μM riboflavin. After the reaction solution was illuminated for 15min at a light intensity of 5000lx, the absorbance was determined at 560 nm with a spectrophotometer(Model UV-4802, Unico, USA). One unit of SOD activity was defined as the amount of enzyme which caused 50% inhibition of the photochemical reduction of NBT.

Guaiacol peroxidase activity was determined following the method of Pütter(1974). Two-hundred microliter of enzyme extract was added to a reaction mixture consisted of 1.5mL of 100mM Potassium-phosphate buffer(pH 7.0), 0.1mL of 80mM guaiacol, and 0.2mL of 100mM H_2O_2. The reaction was started by adding H_2O_2 and the absorbance was measured with a spectrophotometer(Model UV-4802, Unico, USA) against a blank which had an identical mixture except H_2O_2. The enzyme activity was expressed as unit min^{-1} g^{-1} DW, using an extinction coefficient of $26.6mM^{-1}cm^{-1}$.

Lipid peroxidation was estimated from the amount of MDA, an indicator of lipid peroxidation. MDA concentration was determined by measuring the content of 2-thiobarbituric acid-reactive substances in leaf homogenates, prepared in 10% Trichloroacetic acid(TCA) containing 0.5% 2-thiobarbituric acid and heated at 95°C for 25min(Heath and Packer, 1968). The concentration was determined from the adsorption at 532nm and corrected for non-specific turbidity at 600 nm using a spectrophotometer(Model UV-4802, Unico, USA). The concentration of MDA was calculated using a coefficient of absorbance of 155×10^6 cm^2 mol^{-1} and expressed as mol g^{-1} of dry weight(DW).

Proline determination was conducted following the methods described by Bates and Waldren(1973). Approximately 0.2g of frozen leaf segment was ground in 3% sulfosalicylic acid to extract free proline.

Treatment means and standard error of means(SE) were computedwith one-way ANOVA and t-test using the statistical analysis systems(SAS) within each experiment. Post-hoc comparisons were performed using the LSD of means.

Results and Discussion

The average amount of shoot established from the heterogeneous root-zone medium was significantly higher than that of the average of four possible homogeneous media (Table 1). The higher number was attributed to the significant increase in the S+ and S-medium as compared to those in the homogeneous environments, indicating that the offspring ramets

under unfavorable conditions were benefiting from physiological integrations. Alpert et al.(2002) reported similar results of resource sharing in a heterogeneous habitat. Welham et al.(2002) considered that the investment on offspring ramets grown under unfavourable conditions might be an adaptive strategy. Whether the phenomenon observed in this study has an adaptive value in a natural ecosystem or in turfgrass management need further investigation.

Table 1. The amount of shoot established in four-compartment homogeneous and heterogeneous root-zone media from one parent plant located in the center of units.

Medium	Homogeneous[Z]	Heterogeneous	*t*	*P*
P+[Y]	56.8±2.65	60.0±5.52	0.530	0.615
P-	27.6±0.97	33.0±3.34	1.545	0.173
S+	15.8±1.35	22.3±1.89	2.776	0.032
S-	6.1±0.67	9.3±0.85	2.993	0.026
Average	26.56	31.13	2.535	0.044

[Y] P stands for peat, S stands for sieved and acid washed. Watering frequencies were once a week (+) and once in two weeks (-).

[Z] Homogeneous medium consisted one of the four combinations of soil type and water regime. Heterogeneous medium had four compartments each represent one of the four combinations of soil type and water regime.

Single untreated ramet(S_0) showed different SOD activity at 12 h after the application of PEG, and at 60h after stress treatment the connectedramets also showed significant differences(Fig. 1). The pairs with the young ramets treated(O_{ys} and Y_{ys}) showed higher SOD activity than those with old ramets treated(O_{os} and Y_{os}) indicating that young ramets were more sensitive to the stress treatment. SOD activity in S_s started to decrease at 48h(Fig. 1).

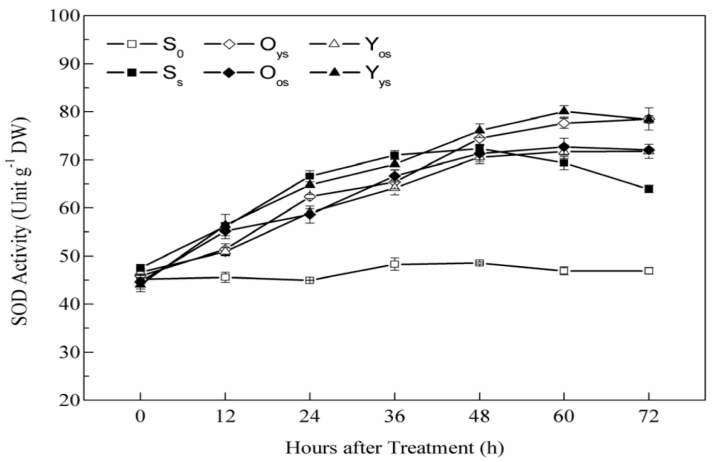

Fig. 1. Time-course of the changes in SOD activity of the leaves collected from the ramets subjected to different treatments: single ramet in Hoagland solution (S_0) and 20% PEG-6000(S_s); and for paired ramets, young ramet in Hoagland solution (Y_{os}) and old ramet in Hoagland solution with 20% PEG-6000 (O_{os}), old ramet in Hoagland solution (O_{ys}) and young ramet in Hoagland solution with 20% PEG-6000 (Y_{ys}). Means of three replicates ± S.E. are shown.

Since the phospholipid membranes are impermeable to reactive O_2^- molecules (Takahashi and Asada, 1983) and SODs are at the first line of defense against O_2^- (Foyer, 1993), the increased SOD of the ramet that was connected to a PEG-treated ramet may have been induced by the signal transferred from the treated ones. It has been reported that hormones may be the early signals in the communication between connected ramets(Qian et al., 2008). For instance, the speed of IAA transportation in plants can be as fast as 48 mm h^{-1}(Gee, 1972). It was reported that maximum Fe-SOD activity was observed 5 h after methyl viologen treatment in Arabidopsis (*Arabidopsis thaliana*) and for an over 41 h time-course(Doge, 1994). The responses of SOD in our study seemed to support the hypothesis that physiological integration between ramets existed.

The activity of G-POD in all treated ramets increased 12h after the initiation of treatment compared with the single untreated ramet as shown in Fig 2. G-POD activity of the O_{ys} and Y_{ys} in paired ramets reached similar levels 36h after treatment. The levels of G-POD in the O_{os} and Y_{os} reached similar levels 48h after the treatment. This suggested that G-POD activity rose faster when the younger ramet was under stress than when the older one was. Connected ramets maintained higher levels of G-POD than the single drought stressed ramet at 48h and after (Fig. 2).

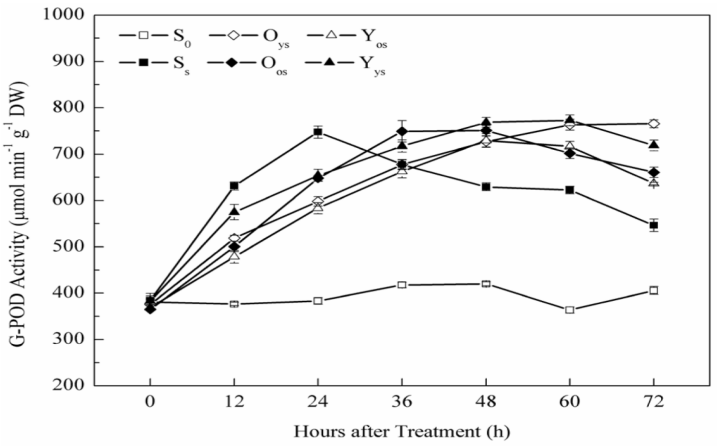

Fig. 2. Time-course of the changes in G-POD activity of the leaves collected from the ramets subjected to different treatments: single ramet in Hoagland solution(S_0) and 20% PEG-6000(S_s); and for paired ramets, young ramet in Hoagland solution(Y_{os}) and old ramet in Hoagland solution with 20% PEG-6000(O_{os}), old ramet in Hoagland solution (O_{ys}) and young ramet in Hoagland solution with 20% PEG-6000(Y_{ys}). Means of three replicates ±S.E. are shown.

In many plant cell defense systems, H_2O_2 generated from O_2^- dismutation by SOD is scavenged by enzymes such as G-POD (Mittler, 2002). The similar pattern of

changes in G-POD and SOD observed in this study may be explained by the closely linked pathway of G-POD and SOD(Fig. 1 and 2).

The content of MDA in the PEG-treated $S_s$showed a significant increase compared with the S_0 24 h after the initiation of stress. The MDA levels in S_s increased throughout the measurement and were significantly higher than all other treatments at 60 h after the stress treatment. At both 60 and 72h after the stress treatment, MDA levels in O_{os} and Y_{ys} were higher than O_{ys} and Y_{os} while there were no differences between O_{os} and Y_{ys}, or O_{ys} and Y_{os}(Fig 3).

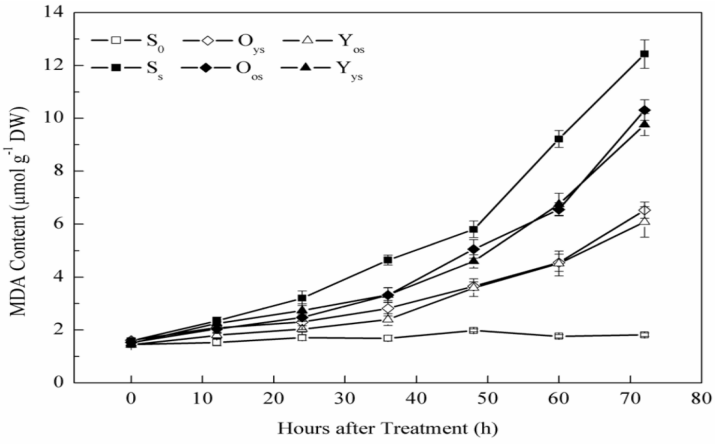

Fig. 3. Time-course of the changes in the content of MDA of the leaves collected from the ramets subjected to different treatments: single ramet in Hoagland solution (S_0) and 20% PEG-6000(S_s); and for paired ramets, young ramet in Hoagland solution (Y_{os}) and old ramet in Hoagland solution with 20% PEG-6000 (O_{os}), old ramet in Hoagland solution (O_{ys}) and young ramet in Hoagland solution with 20% PEG-6000 (Y_{ys}). Means of three replicates ± S.E. are shown.

In other words, MDA levels were higher in stressed ramets than unstressed ramets. The differences between treated ramets and untreated ramets support the general belief that MDA may be an indicator of membrane injury as a result of lipid peroxidation(Jiang and Zhang, 2002). Lipid peroxidation is also important in plant development and in responding to diverse environmental conditions. For example, jasmonates and their octadecanoid precursors are considered as the first oxylipins with an assigned messenger function(Blee, 2002). Compared to S_0, all paired ramets showed elevated MDA levels 60h after the stress treatment. The results indicated some kind of coordinated increase of MDA inconnected ramets.

Significantly higheramount of free proline(Fig. 4) was observed in all treated ramets than the untreated ramets. Although the untreated ramets in the pairs showed higher concentration of free prolinethan the single untreated ramet, the

amounts were less than the treated ramets, indicating that proline was not showing the same trend as in SOD or G-POD but rather similar to the trend shown in MDA.

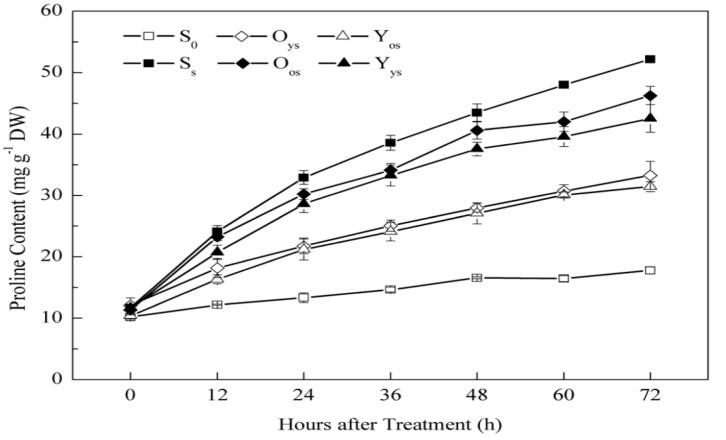

Fig. 4. Time-course of the changes in free Proline acumulation in the leaves collected from the ramets subjected to different treatments: single ramet in Hoagland solution(S_0) and 20% PEG-6000(S_s); and for paired ramets, young ramet in Hoagland solution(Y_{os}) and old ramet in Hoagland solution with 20% PEG-6000(O_{os}), old ramet in Hoagland solution (O_{ys}) and young ramet in Hoagland solution with 20% PEG-6000(Y_{ys}). Means of three replicates ± S.E. are shown.

Mechanisms of stress mediation by proline accumulation have been reported in alleviating cytosolic acidosis (Heuer, 1994), or osmoregulation (Delauney and Verma, 1993 Handa et al., 1996). There has also been suggestion that proline may be a secondary messenger in response to many environmental stresses (Hare et al., 1996). If indeed the proline was acting as a second messenger, it might explain the higher concentrations of proline in the connected ramets unstressed than the single ramets unstressed.

In conclusion, the total amount of biomass produced from heterogeneous root-zone medium was higher than that from homogeneous media for clonal buffalograss with limited water supply. Physiological integrations between connected ramets of buffalograss were detected under heterogeneous water conditions. Lipid peroxidation, antioxidants, and proline were involved in the physiological integration between ramets subjected to different levels of water stress. The mechanisms of integrationmay be different between antioxidants (SOD, G-POD) and MDA or free proline.

국문요약

Buffalograss는 내한, 내서, 그리고 내한발에 우수한 주요 잔디 종 중 하나이다. 다양한 환경에서 buffalograss의 생리학적 조정(integration)을 이해하는 것은 균일한 잔디의 질을 도모하고 경종적 재배방법의 개발에 도움이 된다. 본 연구의 목적은 물 부족의 스트레스 처리에서의 buffalograss의 생리학적 조정과정에서 lipid peroxidation과 산화방지제의 연관성을 평가하였다. 한 실험에서 buffalograss는 네 개의 구분된 칸막이 성장 유닛의 중심에서 재배되었고, 일주일에 한번(+), 일주일에 두 번(-) 관수처리와 모래(S) 또는 피트(P)와를 혼합한 다섯 가지 토양 조합으로 처리하였다(P+S-P-S+, P+P+P+P+, S-S-S-S-, P-P-P-P-, and S+S+S+S+). 그 결과, 균일하게 혼합된 상토에서 생장한 줄기의 수가 네개의 단일 상토에 정착한 것보다 더 많았다. 두 번째 실험에서는 Hoagland 용액(S_o), 또는 20% PEG-6000이 함유된 Hoagland용액(S_s) 안에 하나의 라미트(무성생식체) 혹은 연결된 라마트를 다음과 같은 여러 가지 처리와 비교 실험하였다. 연결된 라미트들의 처리는 Hoagland 용액안의 어린 라미트(Y_{os})와 20%PEG-6000가 함유된 Hoagland 용액안의 성숙한 라미트(O_{os}), Hoagland 용액 단독에 성숙한 라미트(O_{ys}), 20%PEG-6000 함유된 Hoagland 용액안의 어린 라미트(Y_{ys})였다. Lipid peroxidation, antioxidants, proline은 각기 다른 수분 stress 정도에서 라미트들 간의 생리학적 활성을 보여주었다. Superoxide dismutase (SOD), Guaiacol peroxidase (G-POD), malondi aldehyde (MDA), free proline의 활성도 처리 후 시간에 따라 상대적인 생리학적 활성을 보였다.

주요어 : 산화억제, 잔디 관리, 생태, 지방질과산화

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