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Research Article

Phosphorus Significance in Alleviating Oxidative Stress Induced by Drought in Kentucky Bluegrass (*Poa pratensis* L.)

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ABSTRACT

The objective of this study was to determine phosphorus effects on drought stress-induced oxidative stress in Kentucky bluegrass. Drought stress was induced by reducing of water to plants in pots. Two types of phosphorus were applied as potassium phosphate (P) or potassium phosphonate (PA). Application of phosphorus was efficient to ameliorate the adverse effects of drought. Osmotic potential, total chlorophyll and carotenoid content were significantly decreased by drought stress, but was relieved by P or PA application. Superoxide ($O_2^{\bullet-}$) concentration was significantly increased more than 14-fold under drought-stressed plants, was accompanied with increase of hydrogen peroxide (H_2O_2) and lipid peroxidation (MDA). However, malondialdehyde (MDA) was much less in P or PA applied plants under drought stress condition. Activities of catalase (CAT), ascorbate peroxidase (APX) and guaiacol-peroxidase (GPX) were largely increased by drought stress and its increase rate was much higher in P or PA applied plants except APX. These results indicate that drought stress-induced oxidative stress is alleviated by P or PA application due to the increase of activities of antioxidant enzymes.

(**Key words**: Antioxidant enzymes, Drought, Oxidative stress, Potassium phosphate, Potassium phosphonate)

Ⅰ. INTRODUCTION

Kentucky bluegrass is cool-season turfgrass that optimally grows in spring and fall. It is considered as a high-water requirement grass. In Korea, drought stress can occur predominantly in spring and high temperatures throughout the following summer are also unfavorable for growth (Kwon et al., 2016). Drought stress occurred in spring leads to a reduction of Kentucky bluegrass growth in golf course, lawn, parks and grounds. Therefore, it is important to maintain plant growth against drought stress during the management.

During prolonged periods of drought, reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide ions ($O_2^{\bullet-}$), hydroxyl radicals ($OH^{\bullet}$) and hydrogen peroxides (H_2O_2) is accumulated. The generation of ROS is linked to various cellular deterioration including membrane lipid peroxidation, DNA mutation, protein denaturation, and enzyme inactivation (Regoli and Winston, 1999). To scavenge ROS, plants induced natural low molecular mass antioxidants such as glutathione, ascorbate, and carotenoids, and antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidases (POX) and glutathione reductase (GR) (Mittler, 2002). SOD, which is considered as the first defence against ROS, dismutates $O_2^{\bullet-}$ into H_2O_2, and then it converted into non-toxic water by CAT or POXs. Tsugane et al. (1999) reported that the capability of scavenging ROS and reducing their damaging effects may correlate with the drought tolerance of plants. Therefore, the degree of damage depends on the balance between the generation of ROS and their removal by antioxidative systems.

Phosphorus (P) is one of the essential macronutrients in plant tissues. It is a component of key molecules such as nucleic acids, phospholipids, and ATP. P is involved in several key plant functions, including energy storage and transfer, photosynthesis, photorespiration, cell division and multiplication (Lin et al., 2009; Jin et al., 2014). It has been well documented that P application is associated with the enhancement of specific growth factors such as root development, stalk and stem strength, flower formation, seed production, earlier crop maturity and more uniform (Ahn et al., 2005; Jin et al., 2014).

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In addition, exogenous application of P in drought-stressed plants has been found positive effect on plant growth leading to an increase of water-use efficiency, stomatal conductance, and photosynthesis (Garg et al., 2004). On the other hand, P deficiency decreased plant growth in Brassica (Akhtar et al., 2007) and tomato (Ismail and Mohamed, 2010). P starvation induces inhibition of photosynthetic oxygen evolution and thylakoidal ATPase activity due to limited P supply for the photophosphorylation of ADP (Rao, 1997), resulted in photo-oxidative damage of cell components leading to accumulation of $O_2^{\bullet-}$ and lipid peroxidation. Based on these results, P availability might be closely associated with regulation of oxidative damage and plant growth.

In this experiment, we hypothesized that P application may improve drought tolerance of Kentucky bluegrass by modulating antioxidative systems. To test this hypothesis, ROS, lipid peroxidation and antioxidative systems were investigated in Kentucky bluegrass under drought stress conditions with or without P application.

Ⅱ. MATERIALS AND METHODS

1. Plant culture and treatments

Sods of Kentucky bluegrass (*Poa pratensis* L.) were taken from local golf (Muan CC, Chonnam, Korea) and transported to 2 L pot. The pots were cultivated in a greenhouse with a day/night mean temperature of 27/18 °C, and a relative humidity of 65/80%. Natural light was supplemented by metal halide lamps which generated approximately 400 μmol photonsm^{-2} s^{-1} at the canopy height for 16 h day^{-1}. A nutrient solution was continuously supplied to plants as described by Lee et al. (2015). After one month, 200 mL of daily irrigation per pot was supplied to well-watered treatment (control). For drought-stress treatment, 20 mL of daily irrigation containing with or without potassium phosphate (P) or potassium phosphonate (PA) was supplied. After 2 weeks, plants were harvested and separated into shoot and roots. All plant samples were frozen immediately with liquid nitrogen, and then stored in deep freezer (-80 °C) for further analysis.

2. Osmotic potential, chlorophyll and carotenoid content

Leaf sap osmolality was determined with a vapor pressure osmometer (Wescor 5100, Wescor Inc., Logan, UT). For measurement of chlorophyll and carotenoid, fresh leaves were extracted with dimethyl sulfoxide by incubating at 65 °C for 1 h. The absorbances were read at 480, 510, 645 and 663 nm, and calculated using the formulae given by Arnon (1949).

3. Determination of $O_2^{\bullet-}$, H_2O_2 and lipid peroxidation

The leaf $O_2^{\bullet-}$ content was measured as described by Lee et al. (2009) with some minor modifications. The 0.2 g of leaf was homogenized in 50 mM potassium phosphate buffer (pH 7.8) and centrifuged at 12,000 × g at 4 °C for 10 min. The reaction mixture was added with 10 mM NH_2OH-HCl and incubated at 25 °C for 60 min. Then, 17 mM sulfanilamide and 0.01% N-(1-Naphthyl) ethylenediamine were added and incubated at 25 °C for 20 min. The absorbance was read at 530 nm and calculated using $NaNO_2$ standard curve. The H_2O_2 was measured according to Lee et al. (2009). The lipid peroxidation was determined by measuring the concentration of malondialdehyde (MDA), as described previously (Lee et al, 2007).

4. Measurement of antioxidant enzymes activities

Fresh sample (0.5 g) was extracted with 100 mM KPO_4 buffer (pH 7.0) containing 2 mM phenylmethylsulphonyl fluoride, and centrifuged at 14,000 × g at 4 °C for 10 min. The supernatant was used for the measurement of antioxidant enzyme activities as previously described by Lee et al. (2013). The activity of superoxide dismutase (SOD) was determined by measuring its ability to inhibit the photoreduction of nitroblue tetrazolium (NBT). 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. Ascorbate peroxidase (APX) activity was determined by measuring the decrease in absorbance at 290 nm for 1min and calculated using the oxidation of ascorbate (2.8 mM^{-1} cm^{-1}). For guaiacol peroxidase (GPX) activity, the oxidation of guaiacol was estimated by measuring the increase in absorbance at 470 nm for 1 min and the activity was calculated using an absorption for tetraguaiacol (26.6 mM^{-1} cm^{-1}). Catalase (CAT) activity was

assayed by recording a decrease of absorbance at 240 nm for 1min as a result of H_2O_2 degradation (e = 36 mM^{-1} cm^{-1}).

5. Statistical analysis

A completely randomized design was utilized with three replicates for four treatments. Duncan's multiple range test was employed to compare the means of separate replicates. The significant difference was considered at $p<0.05$. All statistical tests were performed using SAS 9.1 (SAS Institute Inc., 2002-2003).

Ⅲ. RESULTS

1. Osmotic potential, total chlorophyll and carotenoid concentration

When plants were exposed to drought stress, osmotic potential (OP) was significantly decreased to −1.63 MPa (-34% compared to control). Potassium phosphate (P)- or potassium phosphonate (PA)-applied plants alleviated the decrease of OP under drought stress condition (Fig. 1). Drought stress decreased by 20.6% and 14.9% in total chlorophyll and carotenoid concentration, respectively, compared to control. No significant difference was observed between P or PA applied drought-stressed plants and control plants (Fig. 2).

2. $O_2^{\bullet-}$ and H_2O_2 content and lipid peroxidation

Superoxide anion ($O_2^{\bullet-}$) content largely increased more than 15-fold by drought stress regardless P or PA application but its

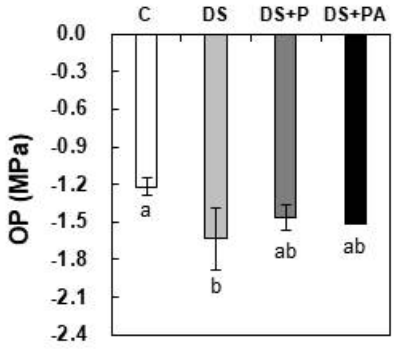

Fig. 1. Osmotic potential (OP) under well-watered (control, C) and drought-stressed (DS) conditions without or with phosphate (DS+P) or phosphonate (DS+PA). Data are shown as mean ± SE for n = 3. Different letters are represented a significant difference between the means at $p<0.05$ according to Duncan's multiple range test.

increased rate was slightly low in PA-applied plants (Fig. 3a). Drought stress slightly increased hydrogen peroxide (H_2O_2) content but not significant in P- or P-applied plants (Fig. 3b). Malondialdehyde (MDA) content, an indicator of lipid peroxidation, in drought stressed plants was remarkedly increased compared to control, whereas it was relieved by PA (1.45-fold higher than control) (Fig. 3c).

3. Activities of antioxidant enzymes

Superoxide dismutase (SOD) activity was the highest in PA-applied plants under drought stress condition (3.0-fold higher than control). Drought stress with P-applied plants (DS+P) and drought alone treated plants increased 1.76- and

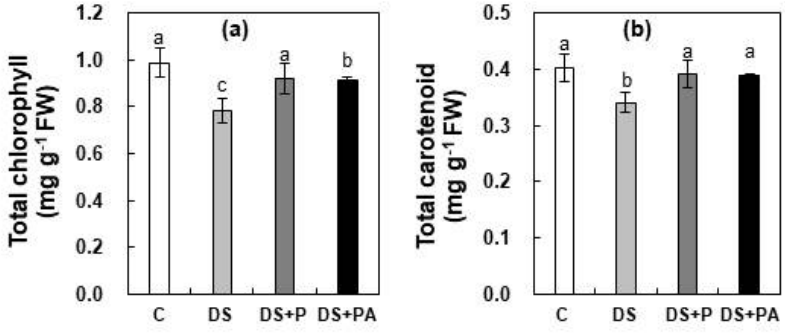

Fig. 2. Total chlorophyll (a) and carotenoid (b) content under well-watered (control, C) and drought- stressed (DS) conditions without or with phosphate (DS+P) or phosphonate (DS+PA). Data are shown as mean ± SE for n = 3. Different letters are represented a significant difference between the means at $p<0.05$ according to Duncan's multiple range test.

2.11-fold compared to control (Fig. 4a). Catalase (CAT) activity was significantly increased by drought stress, especially in P-applied plants (50% higher than control) (Fig. 4b). Ascorbate peroxidase (APX) activity was also increased by drought stress regardless P or PA application (Fig. 4c). Drought stress-induced high guaiacol peroxidase (GPX) activity and its

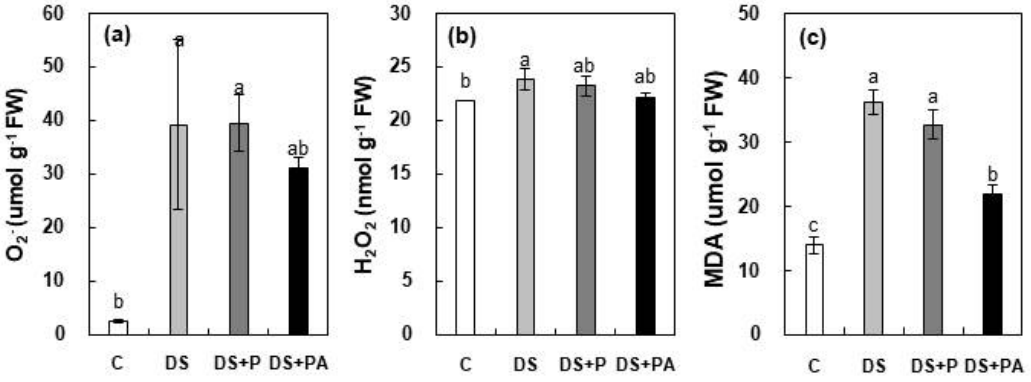

Fig. 3. Superoxide anion ($O_2^{\bullet-}$, a), hydrogen peroxide (H_2O_2, b) and lipid peroxidation (MDA, c) content under well-watered (control, C) and drought- stressed (DS) conditions without or with phosphate (DS+P) or phosphonate (DS+PA). Data are shown as mean ± SE for n = 3. Different letters are represented a significant difference between the means at p<0.05 according to Duncan's multiple range test.

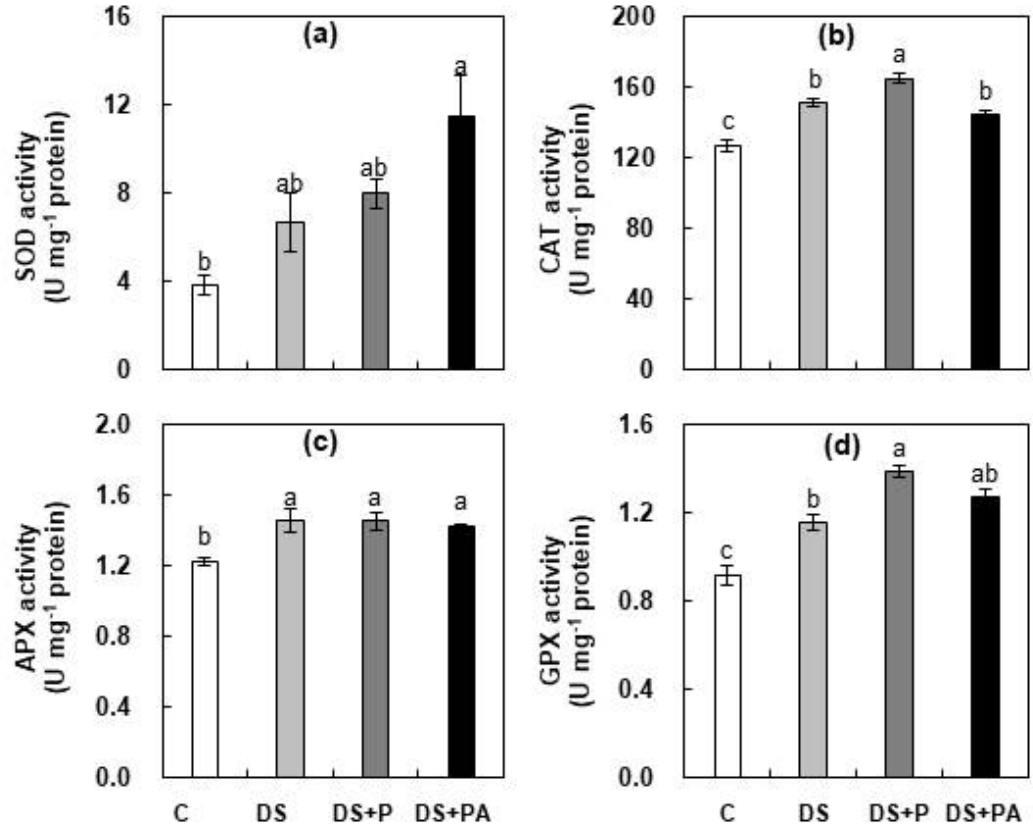

Fig. 4. Activities of superoxide dismutase (SOD, a), catalase (CAT, b), ascorbate peroxidae (APX, c) and guaiacol peroxidase (GPX, d) under well-watered (control, C) and drought-stressed (DS) conditions without or with phosphate (DS+P) or phosphonate (DS+PA). Data are shown as mean ± SE for n = 3. Different letters are represented a significant difference between the means at p<0.05 according to Duncan's multiple range test.

activity was much higher in P (+51% of control) or PA (+40% of control) applied plants than drought stressed (+26.2% of control) plants (Fig. 4d).

Ⅳ. DISCUSSION

As expected, drought stress damaged all the morphophysiological attributes, and pigments such as chlorophyll and carotenoid, but its negative effects were alleviated by P application (Figs. 1 and 2), as observed in drought-stressed Alnus cremastogyne (Tariq et al., 2018). Reduction of OP was the consequence of partial low water absorption and water transport, or high-water loss rate. Given that P nutrition is involved in the promotion of root growth, it is potentially able to obtain greater amounts of soil water (Duursma et al., 2011; Jin et al., 2014). In addition, Singh et al. (2000) reported that P application to white clover in drying soil increased drought tolerance by increasing root hydraulic conductance. These results indicate that P application may improve pigments by enhancing the absorption of water under drought condition.

It has been documented that drought stress-induced stomatal closure inhibited CO_2 uptake by leaves and thus occurred limitation of CO_2 fixation. Reduction of CO_2 fixation results in a decrease of $NADP^+$ regeneration through the Calvin cycle and is related with over reduction of the electron transport chain in chloroplast and mitochondria (Parida et al., 2003). Therefore, over-reduction of photosystem acceptor by photoinhibition condition promotes the formation of $O_2^{\bullet-}$ which converted to H_2O_2 by SOD. The accumulation of ROS such as $O_2^{\bullet-}$ and H_2O_2 in chloroplasts is the main cause of the enhanced lipid peroxidation and chlorophyll bleaching in photo-oxidative stress (Kim and Apel, 2013). In this study, we observed that drought stress resulted in the induction of oxidative stress due to increase of $O_2^{\bullet-}$ and H_2O_2 (Fig. 3a and b), especially $O_2^{\bullet-}$ which showed 20-fold higher than control plants. In addition, an increase of MDA, an indicator of membrane lipid peroxidation, was observed in drought stressed plants (Fig. 3c). However, PA or P application to drought-stressed plants significantly reduced MDA concentration compared to drought-stressed plants, which further improved drought tolerance, as shown in previous study (Tariq et al., 2018).

Accumulated ROS was detoxified by antioxidant enzymes such as SOD, CAT, APX, and GPX which are a general defense mechanism against drought stress. In this study, the results showed significantly enhanced activities of antioxidant enzymes as SOD, GPX, APX, and CAT in drought-stressed plants (Fig. 4). Our results are consistent with other studies that reported the increased antioxidant enzyme activities under drought stress condition in white clover (Lee et al., 2009), tomato (Ahn et al., 2005), and wheat (Bakalova et al., 2004). It has been reported that drought stress tolerance depends on the capability of scavenging ROS and reducing their damaging effects (Tsugane et al., 1999). P or PA application to drought-stressed plants showed a significant increase in activities of GPX and CAT but not APX (Fig. 4). Especially, SOD activity was higher 1.2- and 1.8-fold in P and PA applied plants under drought stress than that of drought-stressed plants, respectively. These results were consistent with a low amount of $O_2^{\bullet-}$ in P- or PA-applied plants under drought stress. In addition, high activities of CAT, APX and GPX are associated with low amount of H_2O_2 and MDA in P or PA applied plants under drought-stressed conditions.

Taken together, drought stress-induced oxidative stress due to the increase of ROS and MDA. To reduce the oxidative stress, antioxidant enzymes (SOD, CAT, APX and GPX) activities were significantly increased under drought-stressed condition. However, drought stress-induced oxidative stress was remarkably alleviated by P nutrition due to the high increase of antioxidant enzymes activities, resulting in a decrease of ROS. It thus concludes that P nutrition participates in improving plant tolerance by mitigating the drought stress-induced oxidative stress.

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