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Effect of Foliar Application of Silicon on Physiological Responses of Chrysanthemum (*Dendranthema ×grandiflorum*) at Two Different Growth Stages

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Abstract

To evaluate the physiological responses of chrysanthemum to silicon spray in non-stress conditions, this study was performed in two separate experiments (at 4 to 5 and 8 to 12-leaf stages) as a factorial randomized completely design with two factors: silicon source (in two levels: sodium silicate and calcium silicate) and the concentration of silicate at five levels (0, 50, 100, 150 and 200 mg L^{-1}) in cocopeat- perlite (1:1 v/v) medium in four replications and two observations. Physiological traits such as catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) activity, proline, malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) content were measured. The results showed that the foliar spray of sodium and calcium silicate increased the activity of CAT, APX and GPX enzymes and MDA, but proline and H_2O_2 were decreased. Generally, in the stage of 8-12 leaves, sodium silicate at high concentrations (150 and 200 mg L^{-1}) was the most effective in increasing antioxidant enzymes activity and reducing proline, MDA and H_2O_2. At 4-5 leaf stage sodium silicate at 100 mg L^{-1} increased antioxidant enzymes activity and calcium silicate at 150 mg L^{-1} reduced proline, MDA and H_2O_2.

Keywords: Ascorbate peroxidase, Calcium silicate, Catalase, Guaiacol peroxidase, Hydrogen peroxide, Sodium silicate.

INTRODUCTION

Chrysanthemum (*Dendranthema* × *grandiflorum*) is one of the most important ornamental plants and its commercial production is often affected by biotic and abiotic stresses (Zhang *et al*., 2005).

After oxygen, silicon is the second most abundant element in the crust of the earth. Plants can use only the $Si(OH_4)$ (Epstein, 1999). Silicon causes resistance to pathogens, biotic and abiotic stresses. Biological stresses cause the accumulation of ROS (Reactive Oxygen Species) including superoxide radicals (O_2^-), hydroxyl radicals (•HO) and hydrogen peroxide (H_2O_2) in plants (Molassiotis *et al*., 2006). Antioxidant enzymes such as guaiacol peroxidase (GPX), ascorbate peroxidase (APX) and catalase (CAT) seems to play an important role in reducing the harmful effects of ROS (Li-Ping *et al*., 2006). Silicon can cause modulation of the oxidative stress, enhance membrane stability and reduce the electrolyte leakage (Karmollachaab *et al*., 2013). A positive relationship between silicon concentration and activity of CAT and APX has been reported in plants (Mohaghegh *et al.*, 2011). Catalase is a major enzyme that can scavenge ROS and inhibit lipid peroxidation, while in plant cells can control the levels of hydrogen peroxide and the damage to cell membranes and chlorophyll. Catalase can control hydrogen peroxide levels in plant cells and seems to play an important role in the photosynthesis process (Khelifa *et al*., 2011). APX is belonging to a group of peroxidases that have been found in chloroplasts, microbodies and cytosols and its main role is to remove the hydrogen peroxide. APX is protecting the plant cells against oxidative stress by using ascorbate as an electron donor (Ghamsari *et al*., 2007; Rosa *et al.*, 2002). GPX is considered as a ROS scavenger because of its tendency to destroy hydrogen peroxide better than catalase (Brigelius-Flohe and Flohe, 2003). MDA is a breakdown product of unsaturated fatty acids and is widely used as a parameter for assay of lipid peroxidation (Mittler, 2002). It is estimated that more than 75% of the MDA is derived from a-Linoleic acid (Weber *et al*., 2004). Accumulation of proline is stimulated in response to environmental stress, osmotic conditions, aging and other factors. Proline plays an important role in osmoregulation (Ahmad and Hellebust, 1988). The aim of this study was to investigate the role of silicon, by foliar application, on the physiological and antioxidant response of chrysanthemum.

MATERIALS AND METHODS

Plant material and growth conditions

This study was performed in two separate experiments. Every experiment had four replications and two observations. In the first experiment (at 4-5-leaf stage of plant growth cycle), there were two factors: the first factor was the two silicone source (sodium silicate and calcium silicate) and the second factor was the concentration of silicate at five levels (0, 50, 100, 150 and 200 mg). The second experiment was done at 8-12-leaf stage with the same treatments as in the first experiment. The medium that was used for the research was a mixture of cocopeat and perlite (1:1 v/v). Plants were grown in a greenhouse in 2 L pots in cocopeat-perlite (1:1 v/v) medium under 17/14°C (day/night) and light intensity of 55-75 µmol $m^{-2}s^{-1}$. Sodium and calcium silicate foliar application was done weekly for 10 weeks. During this period the standard Hoagland nutrient solution (1/4 concentration) was added weekly to the media.

Enzyme extraction and assay

Preparation of plant extracts for determination of CAT, APX and GPX activity was carried out based on Kang and Saltiveit protocol (2002) with slight modifications. 0.5 g of leaves was placed into a cold mortar/pestle. Enzyme extraction was done with 3 ml of Tris buffer (pH =7.5) that contained 3 mM and 1 mM EDTA (and extraction buffer for measuring the activity of APX was consisted of 0.2 mM ascorbate). The homogenates were centrifuged for 20 min at 4°C at 5000 rpm. The supernatant was used as a crude extract for measuring the activity of antioxidant enzymes.

The activity of CAT was measured according to Aebi (1984), APX according to Nakano and Asada (1981) and the GPX according to the protocol of Upadhyaya *et al*. (1985).

To determine the proline content, 0.5 g of fresh leaves were homogenized with 5 ml of 95% ethanol. The upper phase of filtrate was separated and its sediments were washed with 5 ml of 70% ethanol twice and its upper phase added to the previous one. The mixture was centrifuged at 3500 g for 10 min at 4ºC and the supernatant was recovered and the alcoholic extract kept at 4ºC (Paquin and Lechasseur, 1979). One ml of alcoholic extract was diluted with 10 ml of distilled water and 5 ml of ninhydrin (0.125 g ninhydrin, 2 ml of 6 mM NH_3PO_4, 3 ml of glacial acetic acid) and 5 ml of glacial acetic acid was added. The mixture was placed in boiling water bath for 45 min at 100ºC. The reaction was stopped by placing the test tubes in cold water. The samples were vigorously mixed with 10 ml benzene. The light absorption of benzene phase was measured at 515 nm.

Lipid peroxidation in the leaf tissues was determined in terms of malondialdehyde (MDA) content by the thiobarbituric acid (TBA) method, as described by Popham and Novacky (1990). Briefly, 0.2 g of leaf tissue were homogenized in 5 ml of 1% (w: v) trichloroacetic acid (TCA) and then centrifuged at 8000 g for 10 min. One ml of the supernatant was added in to 4 ml of 20 % TCA that contained 0.5 % TBA and the solution was heated for 30 min at 95 °C in water bath. The samples were cooled on ice for 5 min and re-centrifuged for 5 min at 8000 g. Absorbance was measured at 532 nm. Malondialdehyde was calculated according to the following formula and extinction coefficient, $mM^{-1}Cm^{-1}$ 155.

MDA (µmol/g FW) = [A532-A600/155] ×1000

Hydrogen peroxide levels were determined according to the Velikova *et al*., (2000) protocol. 0.1 g fresh weight of leaf tissue was homogenized in 5 ml of 0.1% TCA solution. After centrifugation at 12000 g for 15 min, 0.5 ml of the supernatant was added to the reaction mixture containing 0.5 ml of 10mM potassium phosphate buffer (pH=7.0) and 1 ml of 1M potassium iodide (KI). The reaction mixture was placed at room temperature in the dark for one hour and then the absorbance was determined at 390 nm.

Statistical analysis of experimental data was performed using SAS 9.1 software and the means were compared with Duncan's multiple range test (DMRT) at 5% probability level.

RESULTS AND DISCUSSION

Catalase activity

At both growth stages, the highest activity of CAT was related to sodium silicate. The results showed that 200 and 100 mg L^{-1} silicon were most effective in increasing catalase activity, at 8-12 and 4-5-leaf stage, respectively (Fig. 1 A, B). In 8-12-leaf stage at all levels catalase activity in sodium silicate was higher than calcium silicate. In 8-12-leaf stage with increasing sodium silicate CAT activity was also higher so that the highest catalase activity was observed at 200 mg L^{-1} with 8.574 mM/min. Generally, for increasing catalase activity greater concentration of sodium silicate is needed at 8-12-leaf stage than at 4-5-leaf stage.

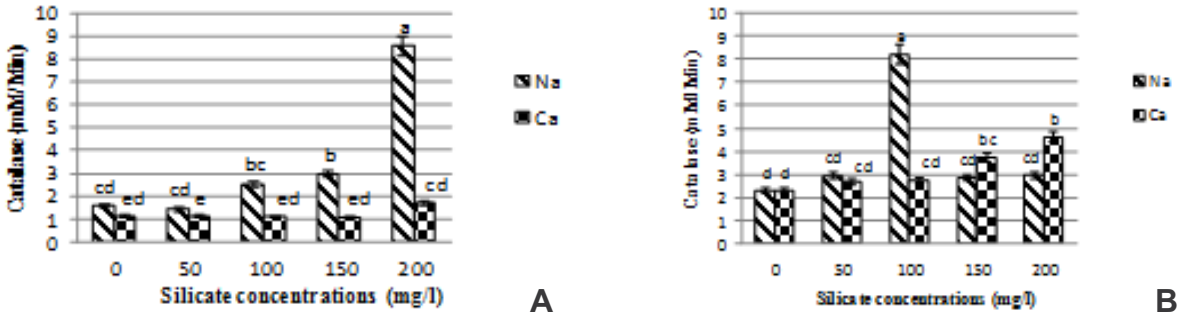

Fig. 1. Effect of sodium and calcium silicate at 8- 12 (A) and 4- 5-leaf stage (B) on catalase activity. Means with the same letter are not significantly different at 5% level of Duncan's test.

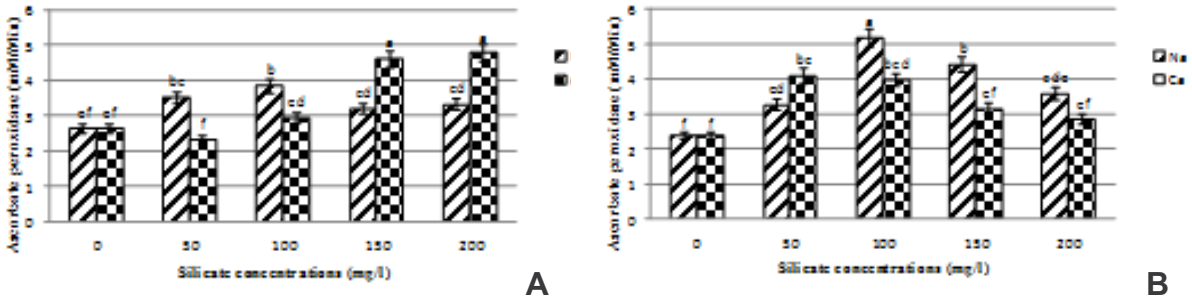

Fig. 2. Effect of sodium and calcium silicate at 8- 12 (A) and 4- 5-leaf stage (B) on ascorbate peroxidase activity. Means with the same letter are not significantly different at 5% level of Duncan's test.

Silicon may increase CAT by affecting catalase production path in peroxisomes. Increasing of CAT production by application of silicon provides conditions for the activation of another antioxidant enzyme such as APX and GPX. Silicon also can increase catalase activity in non-stress conditions. Catalase is able to convert hydrogen peroxide into water and oxygen. Silicon increased catalase activity in wheat (Tale Ahmad and Haddad, 2011), cotton (Moldes *et al.*, 2013) and tomato (Al-Aghabary *et al*., 2004). Catalase activity in plants treated with silicon was 29.2% higher than in silicon-deprived plants (Gang *et al.*, 2008). Liang *et al*. (1996) showed that in barley (salt sensitive and resistant cultivars), silicon increased activity of CAT, SOD and peroxidase in roots.

Ascorbate peroxidase activity

At 8-12-leaf stage calcium silicate was more effective than sodium silicate in increasing APX activity (Fig. 2A) but at 4-5-leaf stage sodium silicate was more effective than calcium silicate (Fig. 2B). By application of sodium silicate at both stages, highest APX rate was obtained at 100 mg L^{-1}. At 4-5-leaf stage the maximum APX amount was obtained at 100 mg L^{-1} sodium silicate with 5.192 mM/min. The most effective concentrations at the 8-12 and 4-5-leaf stages were 200 and 100 mg L^{-1}, respectively.

Silicon likely acted, in the presence of reactive oxygen species, as signal to increase the production of ascorbate peroxidase. Thus, the APX activity was increased by ROS signals; it also may play a role in the suppression of cellular ROS. CAT tendency to hydrogen peroxide (mM) is lower than ascorbate peroxidase (µM). So, APX activity in non-stress conditions; which rate of hydrogen peroxide is little; is more than CAT. APX may act as a signal in the regulation of ROS. APX is the first enzyme in the pathway to remove hydrogen peroxide and accelerated hydrogen peroxide convert into the water. Activity of APX enhanced production of other antioxidant enzymes such as CAT, SOD and glutathione reductase (GR). Application of silicon can increase antioxidant enzymes activity in Helianthus annuus (Moghadam *et al*., 2013), soybean (Miao *et al*., 2010) and wheat (Gharineh and Karmollachaab, 2013). Results of Moldes *et al*. (2013) showed that potassium silicate treatments increased GPX, CAT and APX activity in cotton, in hydroponic systems.

Guaiacol peroxidase activity

The highest level of GPX activity at 8-12 and 4-5-leaf stage was observed in sodium and calcium silicate, respectively (Fig. 3A, B). At 8-12-leaf stage, GPX activity, with sodium silicate at all levels was higher than with calcium silicate. At 4-5-leaf stage, there was no significant difference in GPX activity among sodium silicate concentrations, but in the case of calcium silicate 50 and 100 mg L^{-1} were more effective than the others. GPX is one of the important enzymes that can protect the plant against oxidative stress. Silicon treatment increased SOD, GPX, CAT and POD activity compared to control in sunflower (Moghadam *et al*., 2013). Silicon increases the antioxidant enzymes such as catalase and peroxidase and decreases hydrogen peroxide and malondialdehyde concentrations (Al-Aghabary *et al.*, 2004). Another study showed that silicon increases

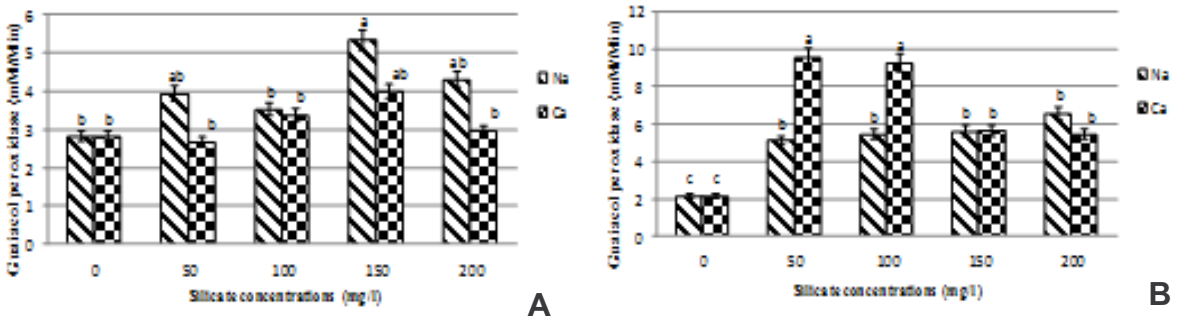

Fig. 3. Effect of sodium and calcium silicate in 8- 12 (A) and 4- 5-leaf stage (B) on guaiacol peroxidase activity. Means with the same letter are not significantly different at 5% level of Duncan's test.

the amount of SOD, CAT, GPX and APX and reduces H_2O_2 and MDA in linen grown in hydroponic conditions (Bharwana *et al*., 2013).

Proline

At 8-12 and 4-5-leaf stages lowest proline levels (0.420 and 0.696 mg g^{-1}), were achieved by sodium and calcium silicate, respectively (Fig. 4A, B). At 8-12-leaf stage at all levels of silicon, proline content in sodium silicate was less than in calcium silicate while at the 4-5-leaf stage the opposite was observed. Proline amount was decreased at both stages with 150 mg L^{-1} sodium and calcium silicate and again increased with 200 mg L^{-1} of both.

Silicon reduces stress effects and activates antioxidant systems in plant. It can reduce the amount of hydrogen peroxide and malondialdehyde that provide the context for proline decrease. Foliar spray of Actisil Hydro Plus decreased free proline in Solomon's seal (Polygonatum multiflorum) in non-stress conditions (Rubinowska *et al*., 2014). Similar results of free proline reduction were obtained silicon treatments Kaya *et al*. (2006) and Tuna *et al.* (2008). Proline content was decreased significantly by application of sodium silicate in bean (Muhammad and Shaheed, 2012). Silicon at 215mM in tomato and 5mM in spinach caused a significant decrease in MDA, membrane permeability, proline and hydrogen peroxide (Hsieh *et al.*, 2002).

Malondialdehyde

At 8-12-leaf stage, silicon reduced MDA at low concentrations but at 4-5-leaf stage at high concentrations. In both 8-12 and 4-5-leaf stages highest MDA level was found in control (0.546 and 0.669 µmol g^{-1} FW, respectively). At 4-5-leaf stage and 8-12-leaf stage the greatest reduction in MDA was caused by 150 mg L^{-1} sodium silicate and 50 mg L^{-1} calcium silicate (Fig. 5A, B). Cell membrane stability is decreased by lipid peroxidation. Silicon by decreasing malondialdehyde causes increasing stability of cell membranes. Silicone increases cell membrane stability through

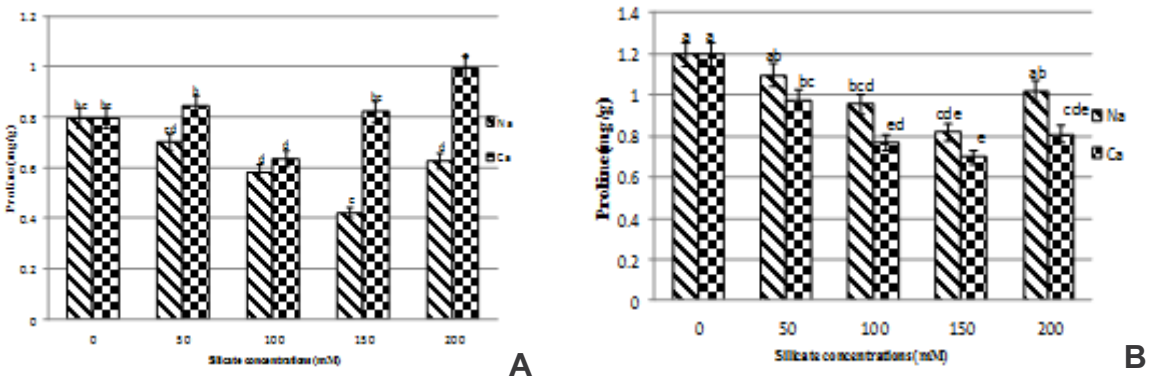

Fig. 4. Effect of sodium and calcium silicate at 8- 12 (A) and 4- 5-leaf stage (B) on proline content. Means with the same letter are not significantly different at 5% level of Duncan's test.

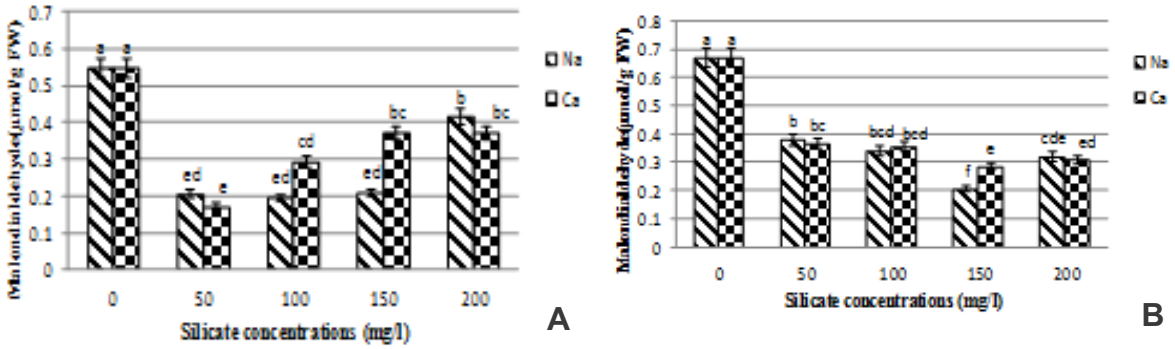

Fig. 5. Effect of sodium and calcium silicate at 8- 12 (A) and 4- 5-leaf stage (B) on malondialdehyde content. Means with the same letter are not significantly different at 5% level of Duncan's test.

changing the ratios of unsaturated and saturated fatty acids which maintained function and integrity of cytoplasmic membrane. Also, silicon increased SOD and APX activity that resulted in reduction in membrane degradation, increased membrane stability and reduced EL (Electrolyte Leakage) and thus increased plant tolerance to stress. Silicon, probably by increasing the potassium ions, protected the cell membrane against damage caused by oxidative stress. The effect of free radical attack on lipids and aldehydes was due to aldehydes including MDA. The increase in this biomarker could be due to the decreased activity of catalase and superoxide dismutase. Silicon decreased malondialdehyde levels compared to control in gerbera cut flowers (Kazemi *et al*., 2012). Silicon and acetyl salicylic applied in carnation caused a decrease in malondialdehyde levels in non-stress conditions (Kazemi *et al*., 2012). Analysis of the cytoplasmic membrane showed that silicon decreased EC (Electrical conductivity) and malondialdehyde in Polygonatum multiflorum (Rubinowska *et al*., 2014). Liang *et al*. (2003) in Hordeum vulgare and Kim *et al*. (2014) in Oryza sativa showed that application of silicon decreased MDA amount. Recent results indicate that silicon increased activity of SOD, POD and CAT but decreased MDA in barley, tomato and maize (Al-Aghabary *et al*., 2004; Mussa 2006; Liang *et al*., 2007). In silicon-treated plants in non-stressed conditions, the activity of SOD, CAT was increased and APX activity slightly decreased (Tale Ahmad and Haddad, 2011).

H_2O_2

At 8-12-leaf stage sodium silicate reduced hydrogen peroxide effectively but at the 4-5-leaf stage calcium silicate was better (Fig. 6A, B). In both stages,150 mg L^{-1} silicate caused a reduction in hydrogen peroxide to its lowest rate. Ascorbate peroxidase, by using two ascorbic acid molecules, converts H_2O_2 to 2 MDHA molecules and water. Silicon by stimulating antioxidant enzymes effects on hydrogen peroxide causes the conversion hydrogen peroxide into water molecules and therefore the decrease in hydrogen peroxide concentration. According to Gunes *et al.* (2008) silicon treatment of sunflower reduced H_2O_2 amount. Sodium silicate causes a decrease in H_2O_2 and MDA in soybean (Miao *et al*., 2010). Zhu *et al*. (2005) reported that Si decreased malondialdehyde and H_2O_2 in wheat. Mussa (2006) showed that silicon increased CAT activity and decreased H_2O_2 in corn.

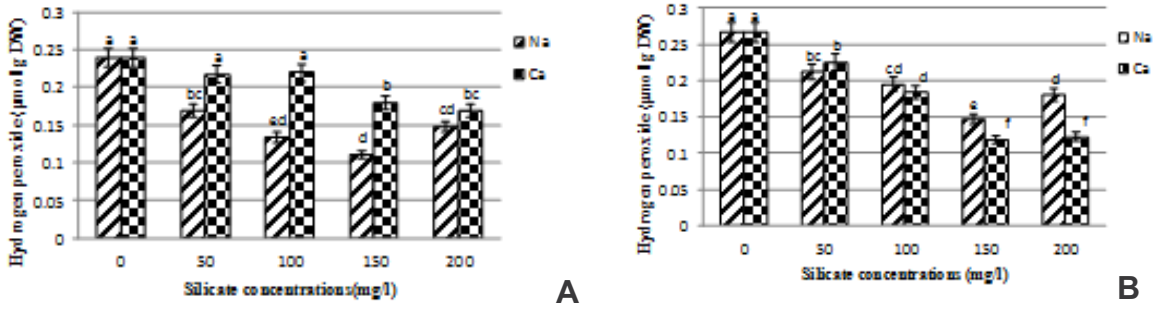

Fig. 6. Effect of sodium and calcium silicate at 8- 12 (A) and 4- 5-leaf stage (B) on H2O2. Means with the same letter is not significantly different at 5% level of Duncan's test.

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