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Changes in Leaf Thickness, Chlorophyll Content, and Gas Exchange of a Landscape Tree, *Xanthostemon chrysanthus*, Treated with Paclobutrazol and Potassium Nitrate

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Abstract. Paclobutrazol (PBZ)(0 g L^{-1}, 0.125 g L^{-1}, and 0.25 g L^{-1}) and potassium nitrate (KNO_3)(0 g $tree^{-1}$, 100 g $tree^{-1}$, and 200 g $tree^{-1}$) were tested on a landscape tree, *Xanthostemon chrysanthus* (F. Muell.) Benth., in an attempt to enhance its stress tolerance under harsh urban conditions. Significant effects on tree height, diameter at breast height, canopy diameter, leaf area, and anatomy of tree leaves and stems in response to PBZ and KNO_3 have been previously reported; in addition to these, the influences on leaf thickness and leaf physiology, including chlorophyll content and gas exchange, are discussed in this study. Relative chlorophyll content was significantly increased with PBZ and/or KNO_3, enhancing leaf greenness. Increased leaf thickness of up to 13.37% at 6 months after treatment with a combination of PBZ and KNO_3 was observed. The presence of PBZ significantly reduced the photosynthetic and transpiration rates and stomatal conductance. Reduced leaf physiological traits combined with thicker leaves would be beneficial for trees to tolerate harsh urban settings. Therefore, a combination of PBZ and KNO_3 is recommended for stress tolerance enhancement of *X. chrysanthus* grown as a landscape tree.

Keywords. Golden Penda; Leaf Physiology; Photosynthesis; Plant Growth Regulator; Urban Tree.

INTRODUCTION

A triazole compound, paclobutrazol, PBZ [(2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl) pentan-3-ol] is widely used in managing ornamental plants. It is an inhibitor of *ent*-kaurenoic oxidase, a key enzyme in the gibberellin (GA) biosynthesis pathway. Application of PBZ reduces GA content in plants, reducing cell elongation and inhibiting growth. As an anti-GA compound, PBZ has been shown to control growth of several plant species (Mohammed et al. 2016; Xia et al. 2018; Ju et al. 2019). Obvious plant responses to PBZ treatment were reduced growth of new shoots, stem diameter, and leaf area; enhanced chlorophyll content; and modified flowering and phytochemical contents in plants (Ahmad Nazarudin et al. 2007; Ahmad Nazarudin 2012; García De Niz et al. 2014). PBZ increased the size of vascular bundles, chloroplasts, and epidermal, mesophyll, and bundle sheath cells in nonwoody plants (Burrows et al. 1992; Gopi et al. 2008; Gao et al. 2011). Only a few studies reported the effects of PBZ on the anatomy of woody plants, including *Syzygium campanulatum* (Ahmad Nazarudin et al. 2007) and *Toona ciliata* (Rodrigues et al. 2016). Plants treated with PBZ were also physiologically affected. In potted *Syzygium myrtifolium*, PBZ reduced both photosynthetic and transpiration rates, though the stomatal conductance was not significantly affected (Ahmad Nazarudin et al. 2012). However, in *T. ciliata*, the gas exchange parameters remained unaffected with PBZ application (Rodrigues et al. 2016). This heterocyclic compound also increases plants' tolerance towards abiotic stresses—for example, drought, flooding, chilling, and salinity. Several other studies reported that PBZ was able to improve water stress tolerance in woody plants such as *Phillyrea angustifolia* (Fernandez et al. 2006) and *Mangifera indica* (Kishor et al. 2009).

The online version of this article has been corrected. See Figure 2.
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Figure 1. ***Xanthostemon chrysanthus*** **planted by the roadside of Metropolitan Batu Park.**

Apart from use of PBZ, correcting issues with culture and remedying nutrient and other deficiencies will typically increase tree growth. However, the application of suitable treatments on landscape trees can be overlooked, hence causing a decline in the growth performance of the trees. For instance, potassium is an essential nutrient and is required for desirable growth of plants. It is essential for the activation of over 80 enzymes throughout the plant (Mengel 2007), improving the plant's ability to withstand extreme conditions such as cold and heat, prolonged drought, and pest and disease attacks (Umar 2006; Thomas and Thomas 2009). Trees planted in urban areas are easily exposed to these growth challenges and need additional attention to ensure their survival.

Although proper application of PBZ and potassium could enhance abiotic stress tolerance in urban trees, studies of managing such trees under local climatic conditions have not been carried out extensively. Thus, *Xanthostemon chrysanthus* (F. Muell.) Benth. (Myrtaceae), commonly known as golden penda, was selected as a model tree for this study. This species is native to tropical northern Australia, New Caledonia, New Guinea, Indonesia, and the Philippines (Sosef et al. 1998). It has also been widely domesticated in Malaysian cities. It is a medium-sized tree that reaches 10 to 15 m in height in its natural habitat. It has bright yellow compound flowers 8 to 12 cm in diameter. Due to its distinctive flowers and dense tree crown, this is a locally preferred species for beautifying residential areas, parks, and roadsides.

This study aimed to determine the effects of PBZ and potassium nitrate (KNO_3) on leaf attributes such as leaf thickness, relative chlorophyll content, and gas exchange in *X. chrysanthus*. Our previous investigation found significant reduction of tree growth in terms of plant height, diameter at breast height, crown diameter, and leaf area of the species in the presence of PBZ (Ahmad Nazarudin et al. 2015). We also observed that the palisade parenchyma thickness was greatly increased after treatment with 0.25 PBZ g L^{-1} + 200 g KNO_3 (Ahmad Nazarudin et al. 2015). However, spongy parenchyma thickness was unaffected by the treatments. Thus, it is also essential to explore the effects of these treatments on leaf thickness and leaf physiology, including chlorophyll content and gas exchange. These assessments were important in order to evaluate the potential of PBZ and KNO_3 to enhance stress tolerance without causing detrimental effects to morphological and physiological traits of the species.

MATERIALS AND METHODS

Study Site and Plant Materials

A total of 81 existing *X. chrysanthus* trees grown at Metropolitan Batu Park, Kuala Lumpur (3°12'49"N, 101°40'43"E) were selected randomly for this study (Figure 1). The trees were planted and maintained by the Kuala Lumpur City Hall (KLCH). For the purposes of the experiment, all trees were selected based on their good growth performance in terms of leaf area index and for being free from pests and diseases. These trees had leaf area index values ranging from 2 to 2.41, heights from 6.01 to 6.91 m, and trunk diameters from 11.02 to 13.70 cm. The trees were planted 1 to 1.5 m away from the road shoulder and 10 m from one another. Nine treatment combinations of PBZ and KNO_3 (control, 100 g KNO_3, 200 g KNO_3, 0.125 g L^{-1} PBZ, 0.125 g L^{-1} PBZ + 100 g KNO_3, 0.125 g L^{-1} PBZ + 200 g KNO_3, 0.25 g L^{-1} PBZ, 0.25 g L^{-1} PBZ + 100 g KNO_3, and 0.25 g L^{-1} PBZ + 200 g KNO_3) with nine replications were laid out in a completely randomized design. No PBZ or KNO_3 was applied to the control trees.

Cultar®, containing 250 g a.i. PBZ L^{-1}, and Krista™ K Plus (13.7:0:46.3) as the source of KNO_3 were used in this study. PBZ was diluted with water prior to application into soil around the root collar of the trees. Each tree received 1 L of PBZ dilution, while the control tree received 1 L of tap water. PBZ was applied once at the beginning of the study (March 2012). On the other hand, each dosage of granular KNO_3 was applied at 3-month intervals. The first application of KNO_3 was done concurrently with PBZ treatment. KNO_3 was applied directly into the soil because the KLCH did not allow foliar spraying for trees grown in the park. Thus, pocket application of KNO_3 was carried out under the tree-canopy drip line to avoid surface runoff during rainy days. Each dosage of KNO_3 was equally divided into 4 portions, each of which was placed approximately equidistant from the others around the canopy drip line. The study was carried out for a year, from March 2012 to March 2013. During the study period, total precipitation received was 3,181.7 mm while the average temperature was between 22.9 and 33.3 °C with relative humidity of 76.4%.

Measurement of Leaf Relative Chlorophyll Content

For the relative chlorophyll content (leaf greenness) investigation, *in situ* measurement was performed by using a portable chlorophyll meter (SPAD-502, Minolta, Japan). The first fully expanded leaves from 3 branches of each tree were selected randomly for the measurement. Relative chlorophyll content was recorded monthly from April 2012 to March 2013 after PBZ and initial KNO_3 treatment. A total of 27 leaves per treatment were measured in monthly sampling.

Determination of Leaf Thickness Increase

Changes in leaf thickness (µm) of *X. chrysanthus* were assessed before treatment (March 2012) and at 6 months after treatment with PBZ and KNO_3 (September 2012). The first fully expanded leaves from 5 randomly selected vegetative branches of each tree were collected for the assessment. Leaf specimens were prepared and observed under a scanning electron microscope (JEOL JSM-5610LV) as described in Ahmad Nazarudin et al. (2015). The thickness increase (%) of the palisade, spongy parenchyma, and leaf was then determined. A total of 45 leaves per treatment were sampled for the assessment.

Measurement of Leaf Gas Exchange

The youngest fully expanded leaves from 3 vegetative branches were selected randomly from each tree for measurement of leaf gas exchange. The measurement was conducted using a portable infrared gas exchange analyzer (Li-6400XT, LICOR, Nebraska, USA). Measurements of photosynthetic rate (µmol m^{-2} s^{-1}), transpiration rate (mmol m^{-2} s^{-1}), and stomatal conductance (mol m^{-2} s^{-1}) were performed between 9:00 a.m. and 11:00 a.m. when photosynthetically active radiation was in the range of 500 to 1200 µmol m^{-2} s^{-1} and carbon dioxide concentration was at 360 to 400 ppm. The measurement was made at ambient humidity and at a maintained temperature of 28 °C. A total of 27 leaves per treatment were measured in each observation. The first and second observations were made in September 2012 and March 2013, respectively.

Statistical Analysis

All data were subjected to 2-way analysis of variance (ANOVA) using Statistical Analysis System version 8.1 (SAS Institute Inc., Cary, NC, USA). Differences between treatment means were compared by using Duncan's multiple range test (DMRT) at 5% level of probability.

RESULTS

Leaf Relative Chlorophyll Content

Initial measurements in April 2012 showed that the leaf relative chlorophyll content was statistically equal among treatments (Table 1). In May 2012, 2 months after treatment, differences in this leaf attribute began to be found. At this stage, the relative chlorophyll content in the control trees was significantly lower than in other treatments. At 6 months after treatment, a difference of about 20% was exhibited between the highest relative chlorophyll content in 0.125 g L^{-1} PBZ + 100 g KNO_3-treated trees and the lowest value in the control trees. However, no significant differences in this leaf attribute were observed among trees treated with PBZ or KNO_3 alone or in combination. These results showed that the relative chlorophyll content in the leaves was markedly augmented with the presence of PBZ and KNO_3, increasing the leaf greenness (Figure 2). This trend of changes in relative chlorophyll content was continuously observed throughout the study period.

Leaf Thickness Increase

Observations via scanning electron microscope discovered that *X. chrysanthus* leaves have one layer of palisade parenchyma, and the spongy parenchyma has a few intercellular spaces (Figure 3). Application of PBZ modified the arrangement of mesophyll cells, making the leaf more compacted (Figure 3c and 3d) as compared to those of the untreated controls (Figure 3a) and KNO_3-treated leaves (Figure 3b). Observation at

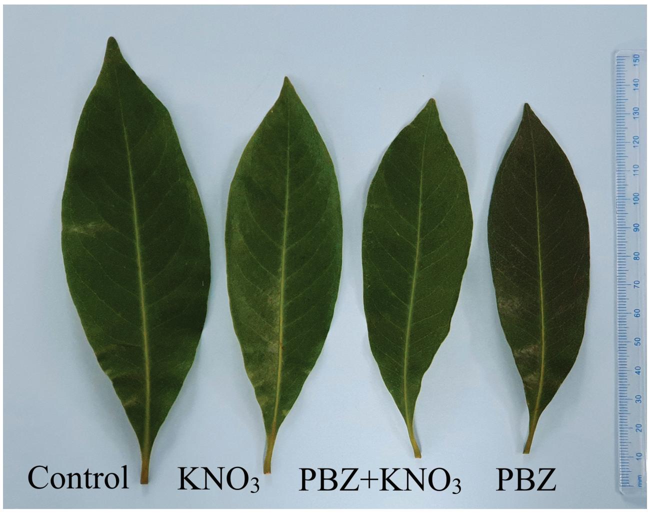

Figure 2. Comparison of leaf size and greenness in *X. chrysanthus* at 6 months after treatment with PBZ and KNO_3.

Table 1. Monthly changes in relative chlorophyll content of *X. chrysanthus* treated with PBZ and KNO_3.

Treatment combination						
PBZ (g L^{-1})	KNO_3 (g)	Apr 2012	May 2012	Jun 2012	Jul 2012	Aug 2012
0	0	41.93 ± 2.69 a	40.92 ± 2.55 b	41.20 ± 1.24 d	41.88 ± 2.41 d	41.21 ± 2.55 c
0	100	42.34 ± 3.16 a	45.57 ± 3.21 a	48.32 ± 4.25 bc	52.24 ± 4.01 ab	50.21 ± 3.26 b
0	200	41.63 ± 3.25 a	45.92 ± 4.99 a	48.55 ± 5.48 bc	50.28 ± 5.14 bc	47.59 ± 3.47 b
0.125	0	40.86 ± 2.43 a	45.17 ± 3.85 a	49.82 ± 2.83 abc	49.88 ± 3.80 bc	49.74 ± 5.39 b
0.125	100	40.92 ± 2.50 a	47.22 ± 5.03 a	52.34 ± 3.43 ab	54.08 ± 4.48 ab	51.16 ± 5.03 ab
0.125	200	40.16 ± 2.47 a	46.91 ± 5.68 a	52.25 ± 4.37 ab	54.38 ± 3.56 ab	52.53 ± 5.78 ab
0.25	0	41.88 ± 1.82 a	46.81 ± 2.17 a	50.67 ± 4.15 abc	53.61 ± 3.09 ab	51.82 ± 3.31 ab
0.25	100	40.30 ± 2.90 a	46.78 ± 3.38 a	50.82 ± 4.31 abc	54.21 ± 3.47 ab	51.92 ± 5.55 ab
0.25	200	41.48 ± 3.49 a	45.71 ± 5.94 a	48.82 ± 4.46 abc	54.35 ± 4.65 ab	52.16 ± 5.25 ab

Means followed by the same letter(s) within column do not differ by DMRT (2-way ANOVA, $p < 0.05$); Mean ± standard deviation; $n = 27$/treatment.

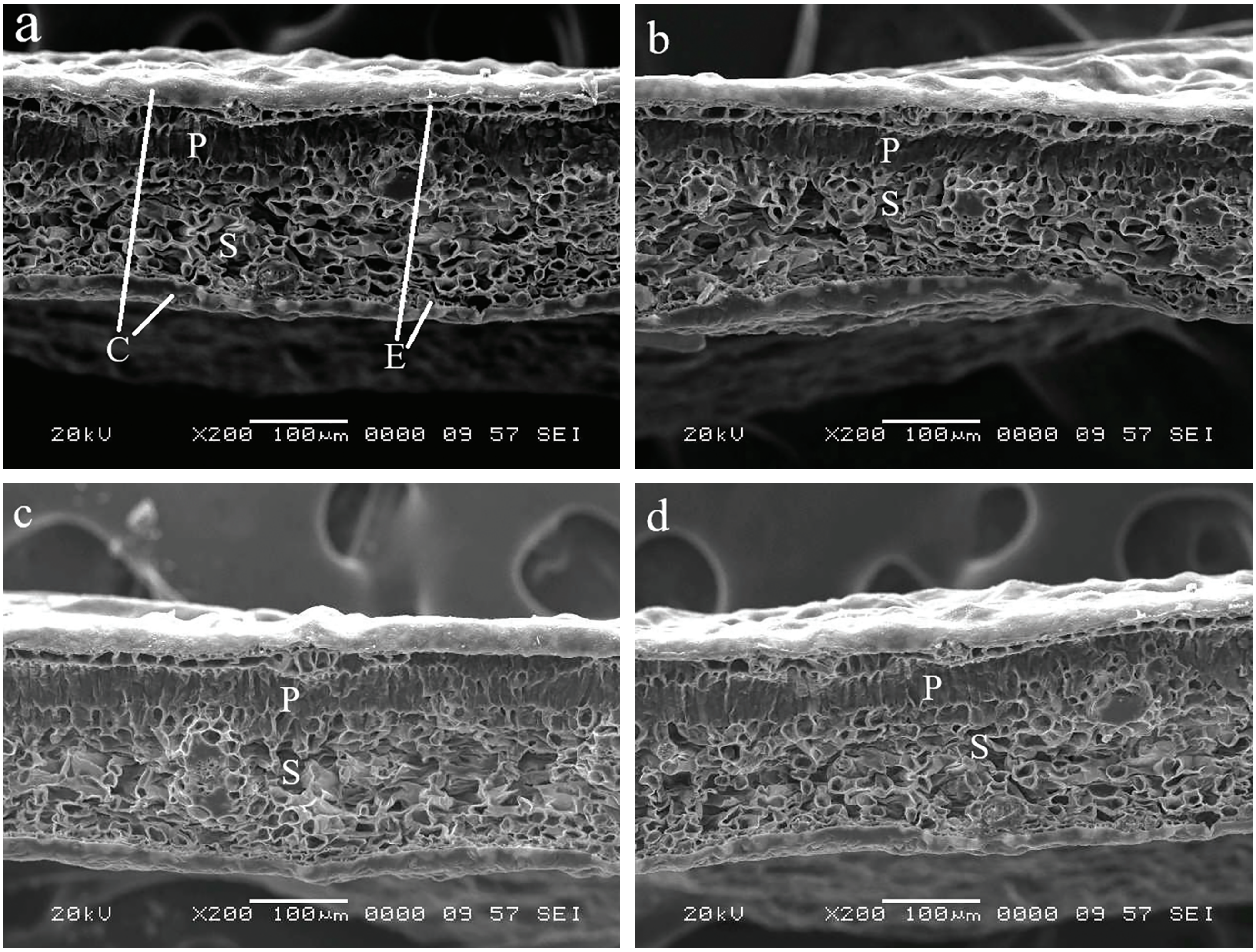

Figure 3. Micrographs of *X. chrysanthus* leaves at 6 months after treatment with PBZ and KNO_3. (a) Untreated control leaf; (b) KNO_3-treated leaf; (c) PBZ-treated leaf; (d) PBZ + KNO_3-treated leaf. Palisade parenchyma (P); Spongy parenchyma (S); Epidermis (E); Cuticle (C).

Relative chlorophyll content						
Sep 2012	**Oct 2012**	**Nov 2012**	**Dec 2012**	**Jan 2013**	**Feb 2013**	**Mar 2013**
41.28 ± 1.98 b	41.23 ± 1.22 b	41.09 ± 2.62 b	40.14 ± 4.30 b	41.06 ± 1.69 b	39.48 ± 1.99 c	39.44 ± 2.05 b
49.72 ± 3.81 a	48.61 ± 2.00 a	49.23 ± 3.69 a	48.97 ± 4.39 a	49.21 ± 5.26 a	48.28 ± 3.70 ab	49.70 ± 5.04 a
47.48 ± 7.03 a	48.70 ± 5.77 a	47.53 ± 2.99 a	48.76 ± 4.73 a	46.72 ± 6.30 a	47.37 ± 4.73 ab	47.60 ± 3.11 a
49.92 ± 4.72 a	48.62 ± 3.64 a	49.63 ± 3.82 a	51.44 ± 5.49 a	50.44 ± 3.01 a	48.54 ± 3.38 ab	47.92 ± 3.33 a
51.63 ± 4.64 a	53.06 ± 4.13 a	51.27 ± 3.94 a	52.82 ± 7.20 a	49.63 ± 4.90 a	50.58 ± 5.50 ab	48.96 ± 4.86 a
51.30 ± 3.36 a	51.12 ± 4.54 a	50.79 ± 6.37 a	48.89 ± 3.14 a	50.79 ± 6.79 a	50.38 ± 2.84 ab	50.21 ± 2.88 a
50.93 ± 5.20 a	51.29 ± 3.41 a	49.09 ± 3.50 a	51.78 ± 4.51 a	49.09 ± 4.71 a	50.26 ± 5.21 ab	49.02 ± 5.60 a
50.07 ± 2.81 a	52.09 ± 5.75 a	51.66 ± 6.00 a	51.71 ± 3.97 a	51.43 ± 4.40 a	49.63 ± 3.79 ab	50.32 ± 3.83 a
51.29 ± 6.47 a	49.86 ± 3.94 a	49.54 ± 2.84 a	51.60 ± 5.88 a	50.22 ± 4.76 a	51.58 ± 4.94 a	50.94 ± 5.09 a

Table 2. Thickness increase of palisade and spongy parenchyma in *X. chrysanthus* at 6 months after treatment with PBZ and KNO_3.

Treatment combination		Palisade parenchyma thickness increase (%)	Spongy parenchyma thickness increase (%)
PBZ (g L^{-1})	KNO_3 (g)		
0	0	2.49	1.27
0	100	14.69	1.58
0	200	16.79	1.12
0.125	0	36.32	1.37
0.125	100	42.38	−0.74
0.125	200	44.43	−0.06
0.25	0	37.41	0.67
0.25	100	44.31	−1.02
0.25	200	51.12	−1.18

Table 3. Changes in leaf thickness of *X. chrysanthus* at 6 months after treatment with PBZ and KNO_3.

Treatment combination		Leaf thickness (µm)		Leaf thickness increase (%)
PBZ (g L^{-1})	KNO_3 (g)	0 months after treatment	6 months after treatment	6 months after treatment
0	0	185.40 ± 9.69 a	187.08 ± 8.50 d	0.91
0	100	184.19 ± 6.26 a	193.21 ± 10.98 c	4.89
0	200	184.21 ± 8.20 a	193.18 ± 8.69 c	4.87
0.125	0	185.39 ± 5.65 a	203.77 ± 9.48 b	9.91
0.125	100	184.64 ± 3.91 a	206.90 ± 8.36 ab	12.06
0.125	200	185.41 ± 6.64 a	207.14 ± 8.85 ab	11.72
0.25	0	184.62 ± 6.34 a	203.44 ± 10.26 b	10.19
0.25	100	184.18 ± 4.78 a	206.84 ± 7.79 ab	12.30
0.25	200	185.44 ± 3.68 a	210.23 ± 7.11 a	13.37

Means followed by the same letter(s) within column do not differ by DMRT (2-way ANOVA, $p < 0.05$); Mean ± standard deviation; $n = 45$/treatment.

6 months after treatment with 0.25 g L^{-1} PBZ + 200 g KNO_3 showed an increase of palisade parenchyma thickness of up to 51.12%, while the controls only showed a 2.49% thickness increase over measurements recorded at the start of the study (Table 2). It has been proven in our previous investigation that trees treated with the combination of PBZ and KNO_3 resulted in greater values of palisade parenchyma thickness as compared to those treated with PBZ or KNO_3 alone (Ahmad Nazarudin et al. 2015). At the same time, treatments with KNO_3 alone (100 g KNO_3 and 200 g KNO_3) have a thicker palisade parenchyma as compared to the control trees at 6 months after treatment (Ahmad Nazarudin et al. 2015). As a consequence of the increased palisade parenchyma layer thickness, the leaf thickness of *X. chrysanthus* also increased (Table 3). In this study, the leaf thickness increase in the trees treated with 0.25 g L^{-1} PBZ + 200 g KNO_3 was 13.37%, while there was only a 0.91% increase for this parameter among the controls (Table 3). Application of PBZ or KNO_3 alone increased the leaf thickness by approximately 10% and 4.9%, respectively.

Leaf Physiological Performance

Photosynthesis, transpiration, and stomatal conductance were significantly different among treatments at 6 and 12 months after the treatment (Table 4). The highest photosynthetic rate was found in the control trees. This study showed equal response in photosynthetic rate between trees treated with different amounts of PBZ alone, and between trees treated with different amounts of KNO_3 alone. There was

Table 4. Physiological response of *X. chrysanthus* at 6 and 12 months after treatment with PBZ and KNO_3.

Treatment combination		Photosynthetic rate ($\mu mol\ m^{-2}s^{-1}$)		Transpiration rate ($mmol\ m^{-2}s^{-1}$)		Stomatal conductance ($mol\ m^{-2}s^{-1}$)	
PBZ ($g\ L^{-1}$)	KNO_3 (g)	6 months after treatment	12 months after treatment	6 months after treatment	12 months after treatment	6 months after treatment	12 months after treatment
0	0	6.56 ± 1.07 a	6.02 ± 0.93 a	3.04 ± 0.80 a	2.72 ± 0.56 a	0.14 ± 0.03 a	0.14 ± 0.07 a
0	100	5.28 ± 0.60 b	5.40 ± 0.74 b	2.18 ± 0.59 b	1.90 ± 0.37 b	0.11 ± 0.08 a	0.12 ± 0.06 a
0	200	5.50 ± 0.69 b	5.07 ± 0.76 b	2.01 ± 0.53 b	1.95 ± 0.51 b	0.11 ± 0.08 a	0.11 ± 0.07 a
0.125	0	3.82 ± 0.61 cde	3.53 ± 0.63 c	0.78 ± 0.12 c	0.76 ± 0.14 c	0.05 ± 0.02 b	0.05 ± 0.02 b
0.125	100	3.86 ± 0.62 cd	3.58 ± 0.90 c	0.84 ± 0.11 c	0.76 ± 0.15 c	0.05 ± 0.02 b	0.03 ± 0.01 b
0.125	200	3.92 ± 0.93 cd	3.73 ± 0.93 c	0.77 ± 0.13 c	0.74 ± 0.16 c	0.04 ± 0.02 b	0.03 ± 0.01 b
0.25	0	3.25 ± 1.17 de	3.23 ± 0.82 c	0.76 ± 0.13 c	0.72 ± 0.14 c	0.05 ± 0.03 b	0.04 ± 0.03 b
0.25	100	3.55 ± 0.90 de	3.31 ± 0.55 c	0.76 ± 0.12 c	0.74 ± 0.13 c	0.02 ± 0.01 b	0.03 ± 0.01 b
0.25	200	3.42 ± 0.58 de	3.27 ± 0.38 c	0.75 ± 0.13 c	0.74 ± 0.11 c	0.02 ± 0.01 b	0.03 ± 0.02 b

Means followed by the same letter(s) within column do not differ by DMRT (2-way ANOVA, $p < 0.05$); Mean ± standard deviation; $n = 27$/treatment.

also a statistically equal photosynthetic rate among trees that had combined treatments of PBZ and KNO_3. However, comparison among different treatments revealed that the presence of PBZ resulted in lower rates of photosynthesis than in trees treated with KNO_3 alone and the control trees. At 6 months after treatment, the highest photosynthetic rate was 6.56 $\mu mol\ m^{-2}s^{-1}$ in the control trees, while trees treated with 0.25 $g\ L^{-1}$ PBZ had the lowest photosynthetic rate (3.25 $\mu mol\ m^{-2}s^{-1}$), almost a twofold difference. At this stage, the highest transpiration rate was recorded in the control trees, while the lowest was demonstrated by 0.25 $g\ L^{-1}$ PBZ + 200 g KNO_3-treated trees (Table 4). The transpiration rate of the control trees was higher by 75.33% than those trees treated with 0.25 $g\ L^{-1}$ PBZ + 200 g KNO_3. Comparison between different rates of PBZ or KNO_3 tested alone, and among combined treatments of both compounds gave no differences in terms of transpiration rate. This study suggested that the transpiration rate of the trees markedly decreased in the presence of PBZ. As for stomatal conductance, no significant differences were demonstrated between the two treatment levels of PBZ alone or the two levels of KNO_3 alone. Nor were significant differences shown among trees treated with combined treatments of PBZ and KNO_3. However, the presence of PBZ triazole significantly reduced the stomatal conductance, resulting in lower values as compared to those of the KNO_3-treated trees and the controls (Table 4). These treatments showed similar responses in photosynthetic rates, transpiration rates, and stomatal conductance at 12 months after treatment as they did at 6 months.

DISCUSSION

Urban trees are exposed to unfavorable biotic and abiotic stresses such as hot and cold temperatures, drought, poor soil conditions, and pests and diseases. These factors, which can lead to a decline in growth performance, are among the challenges facing trees in urban areas. Thus, maintenance approaches that enhance growth of urban trees are indeed essential. This study explored one possible technique for mitigating urban stresses in an existing landscape tree, *X. chrysanthus*, by looking into the possibility of using PBZ and KNO_3 to improve the tree's tolerance towards those challenges.

Our previous study indicated that the presence of PBZ suppressed leaf expansion, producing smaller leaf area as compared to those of other treatments (Ahmad Nazarudin et al. 2015). However, no abnormal leaf formation (leaf discoloration or anomalous growth) was observed (Figure 2), showing that the dosages of PBZ used in this study were appropriate for the species. The reduced size of PBZ-treated leaves may be associated to PBZ's inhibitory action on GA biosynthesis, which is involved in cell elongation (Ahmad Nazarudin et al. 2015). Several other studies also showed reduced leaf area following PBZ treatment (Yeshitela et al. 2004; Chorbadjian et al.

2011; Ahmad Nazarudin et al. 2012). In addition, a darker green color in leaves was exhibited as a response to PBZ and KNO_3 treatments (Figure 2). This was due to enhanced relative chlorophyll content associated with smaller leaf area, which further intensified the foliage color. Increased relative chlorophyll content as a response to PBZ was consistently reported in various plant species including *Hibiscus rosa-sinensis* (Ahmad Nazarudin 2012), *Lagerstroemia indica* (Mohammed et al. 2016), and *Paeonia lactiflora* (Xia et al. 2018).

Furthermore, the combination of PBZ and KNO_3 produced relatively thicker leaves as compared to those treated with either PBZ or KNO_3 alone. This study corroborates findings by Gopi et al. (2008) and Gao et al. (2011) which indicated that PBZ increased the leaf thickness of *Amorphophallus campanulatus* and *Triticum aestivum*, respectively. Increased leaf thickness also resulted from inhibition of leaf size in combination with further thickening of the palisade mesophyll cells. An enhanced palisade layer in other woody species such as *S. campanulatum* (Ahmad Nazarudin et al. 2007) and *T. ciliata* (Rodrigues et al. 2016) following PBZ application has also been documented. A previous study has also shown that PBZ resulted in thicker leaves in *Chrysanthemum* due to an additional layer of palisade, although individual palisade cells were shorter (Burrows et al. 1992). In the present study, observation through a scanning electron microscope proved that the *X. chrysanthus* leaf retained a single layer of palisade parenchyma. Therefore, the enhancement of leaf thickness in this species was actually influenced by the increased palisade parenchyma thickness rather than the additional layer of the palisade. Other than PBZ, potassium was also proven to increase chlorophyll content and leaf thickness in *Gossypium hirsutum* (Akhtar et al. 2009). Enhanced leaf thickness due to PBZ and KNO_3 as observed in this study might be beneficial for *X. chrysanthus* to prevent biotic and abiotic stresses. Leaf thickness plays an essential role in plant functioning and relates to a species' strategy for resource acquisition and use (Vile et al. 2005). Increased tissue thickness in the leaf enabling enhanced water-holding capacity with the presence of potassium helped improve metabolic activities and production of photosynthates like carbohydrates and proteins and their translocation to respective sinks. For example, Australian desert plants have relatively thick, long-lived leaves (Wright and Westoby 2002) and grow in soils that are uncommonly low in nutrients (Morton et al. 2011). Under such conditions, prolonging the leaf life span could be achieved through the production of leaves that are not only structurally tough and herbivore resistant, but also resistant to thermal damage (Leigh et al. 2012). In addition, physical modification of leaves as a response to PBZ, for example thicker leaves, smaller stomatal pores, and an expanded epicuticular wax layer on the leaf surface, may provide additional protections against various fungal, bacterial, and insect infestations (Chaney 2005).

In the present study, a significant reduction in photosynthetic rates, transpiration rates, and stomatal conductance was demonstrated in the presence of the PBZ compound. Similar results of reduced photosynthetic rate as a response to PBZ treatment were also reported in *L. indica* (Mohammed et al. 2016) and *S. myrtifolium* (Ahmad Nazarudin et al. 2012). This could be the indirect effect of modified cell arrangement in the leaves caused by PBZ, which eventually restricted gas exchange. It was proven in our previous experiment that the parenchyma cells of the PBZ-treated leaves were tightly packed because the decreased leaf size forced these tissues into such an arrangement (Ahmad Nazarudin et al. 2015). Fortunately, reduction in the transpiration rate and stomatal conductance could protect the plant against abiotic stresses related to water limitations or drought incidents. It could possibly decrease the amount of water lost through stomata. Fletcher et al. (2000) stated that triazole-treated plants had lower transpiration, required less water, and were able to adapt better to drought than untreated plants. In addition, KNO_3, which supplies potassium, may also be beneficial in other aspects of plant biochemical processes, such as activating enzymes, regulating osmosis, and transporting photosynthates in the plant. As a consequence, the treated trees have greater tolerance to environmental stresses. Thus, PBZ and KNO_3 may help protect this species when it is planted in harsh urban areas that expose it to the above-mentioned limitations.

CONCLUSION

In conclusion, the presence of PBZ produced a more compressed arrangement of cells in the leaves. Darker green leaves were observed due to enhanced relative chlorophyll content following PBZ and KNO_3 treatment. The combination of PBZ and KNO_3 also dramatically increased the leaf thickness of *X. chrysanthus*. In addition, reduced physiological attributes of photosynthetic and transpiration rates and stomatal conductance were found in the presence of PBZ. Reduction of the physiological capacity would reduce the amount of water loss through transpiration and help to enhance the water-holding ability of the leaves. Thus, PBZ would be beneficial for trees planted in harsh urban settings, which usually experience water constraints due to improper soil conditions, drought, and heat. This study recommends the combination of PBZ and KNO_3 as one of the treatments for *X. chrysanthus* under local climatic conditions where improved tolerance to environmental stresses is desired.

LITERATURE CITED

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Résumé. Le paclobutrazole (PBZ)(0 g L^{-1}, 0,125 g L^{-1}, et 0,25 g L^{-1}) et le nitrate de potassium (KNO_3)(0 g $arbre^{-1}$, 100 g $arbre^{-1}$, et 200 g $arbre^{-1}$) ont été testés sur un arbre d'ornement, *Xanthostemon chrysanthus* (F. Muell.) Benth. afin d'améliorer sa tolérance au stress dans les conditions urbaines difficiles. Des effets significatifs sur la hauteur des arbres, le diamètre à hauteur de poitrine, l'étendue de la ramure, la surface foliaire et la structure des feuilles et des tiges des arbres ont déjà été signalés en réaction aux PBZ et KNO_3; en outre de ces effets, leur influence sur l'épaisseur et la physiologie des feuilles, notamment la teneur en chlorophylle et les échanges gazeux, sont examinées dans cette recherche. La teneur relative en chlorophylle a été augmentée de manière significative avec du PBZ et/ou du KNO_3, ce qui a amélioré la coloration verte des feuilles. Une augmentation de l'épaisseur des feuilles allant jusqu'à 13,37%, six mois après le traitement avec une combinaison de PBZ et KNO_3, fut constatée. La présence de PBZ a réduit de manière significative les taux de photosynthèse, de transpiration et de conductance stomatale. La réduction de caractéristiques physiologiques des feuilles, combinée à des feuilles plus épaisses, permettrait aux arbres de mieux tolérer les conditions urbaines difficiles. Par conséquent, il est recommandé d'utiliser une combinaison de PBZ et KNO_3 afin d'améliorer la résistance au stress lorsque *X. chrysanthus* est utilisé comme arbre d'ornement.

Zusammenfassung. In einem Versuch zur Verbesserung der Stresstoleranz eines Landschaftsbaums, *Xanthostemon chrysanthus* (F. Muell.) Benth., wurden Paclobutrazol (PBZ)(0 g L^{-1}, 0,125 g L^{-1}, und 0,25 g L^{-1}) und Kaliumnitrat (KNO_3)(0 g $Baum^{-1}$, 100 g $Baum^{-1}$, und 200 g $Baum^{-1}$) unter rauen, städtischen Bedingungen getestet. Signifikante Auswirkungen auf die Baumhöhe, den Brusthöhendurchmesser, den Kronen-Durchmesser, die Blattfläche und die Anatomie der Blätter und Stämme des Baumes wurden zuvor als Reaktion auf PBZ und KNO_3 berichtet. Zusätzlich zu diesen werden in dieser Studie die Einflüsse auf die Blattdicke und die Blattphysiologie, einschließlich des Chlorophyll-Gehalts und des Gasaustauschs, diskutiert. Der relative Chlorophyll-Gehalt wurde durch PBZ und/oder KNO_3 signifikant erhöht, wodurch die Blattgrünheit gesteigert wurde. Eine erhöhte Blattdicke von bis zu 13,37% wurde 6 Monate nach der Behandlung mit einer Kombination aus PBZ und KNO_3 beobachtet. Die Anwesenheit von PBZ reduzierte signifikant die Photosynthese- und Transpirationsraten sowie die stomatäre Leitfähigkeit. Reduzierte blattphysiologische Eigenschaften in Kombination mit dickeren Blättern wären für Bäume vorteilhaft, um die raue städtische Umgebung zu tolerieren. Daher wird empfohlen, eine Kombination aus PBZ und KNO_3 zur Verbesserung der Stresstoleranz von *X. chrysanthus* als Landschaftsbaum zu verwenden.

Resumen. Se probaron Paclobutrazol (PBZ)(0 g L^{-1},0.125 g L^{-1}, y 0.25 g L^{-1}) y nitrato de potasio (KNO_3)(0 g $árbol^{-1}$, 100 g $árbol^{-1}$, y 200 g $árbol^{-1}$) en un árbol del paisaje, *Xanthostemon chrysanthus* (F. Muell.) Benth., en un intento de mejorar su tolerancia al estrés bajo condiciones urbanas críticas. Los efectos significativos en la altura del árbol, diámetro a la altura del pecho, diámetro de la copa, área de la hoja, y la anatomía de las hojas y tallos se han divulgado previamente en respuesta a PBZ y KNO_3; además de éstos, las influencias en el grosor de las hojas y la fisiología de las hojas, incluyendo el contenido de clorofila y el intercambio de gases, se discuten en este estudio. El contenido relativo de clorofila se incrementó significativamente con PBZ y/o KNO_3, mejorando el verdor de las hojas. Se observó un aumento del espesor de la hoja de hasta el 13,37% a los 6 meses después del tratamiento con una combinación de PBZ y KNO_3. La presencia de PBZ redujo significativamente las tasas fotosintéticas y de transpiración y la conductancia estomatal. La reducción de los rasgos fisiológicos de las hojas combinadas con hojas más gruesas sería beneficioso para los árboles al tolerar el duro entorno urbano. Por lo tanto, se recomienda utilizar una combinación de PBZ y KNO_3 para mejorar la tolerancia al estrés de *X. chrysanthus* cultivado como árbol urbano.