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Full Paper

Effects of uniconazole on stress resistance indices, metabolite content and endogenous hormones of potted *Paeonia lactiflora* 'Dafugui'

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Abstract: In order to use plant growth regulators more efficiently and rationally, we conducted a comprehensive study on the dwarfing effect of uniconazole on *Paeonia lactiflora*. The leaves of 'Dafugui' cultivar were sprayed with uniconazole at different concentrations (0, 20, 40 and 60 mg·L^{-1}) to explore its effects on physiological and biochemical characteristics of the potted plant. Compared with control, uniconazole (40 mg·L^{-1}) increased the activities of superoxide dismutase, peroxidase and catalase, but had no significant effect on the accumulation of malondialdehyde. Soluble protein content was significantly highest in all four growth stages. Soluble sugar, sucrose and reducing sugar contents were significantly highest at the full-flowering stage. The treatment also decreased indole-3-acetic acid, gibberellic acid and *trans*-zeatin riboside contents of leaves by 5.78%, 4.82% and 22.03% respectively, but increased abscisic acid content by 67.58%. This suggests that the stress resistance of the potted plant was enhanced.

Keywords: *Paeonia lactiflora*, herbaceous peony, uniconazole, growth retardant

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INTRODUCTION

Paeonia lactiflora of the Paeoniaceae family is a perennial herbaceous plant which has a long history of cultivation in China. *Paeonia* flowers are beautiful and deeply revered by the Chinese people and globally [1]. The rapid development of the garden flower industry in China is a result of the demand for high-quality flowers and the popularity of planting potted flowers, especially during holidays [2]. However, differences in environmental conditions such as light, temperature, water and fertiliser during pot plant cultivation, compared with conventional open-field cultivation, often lead to the poor performance of potted *P. lactiflora*, such as irregular or excessive growth and development of thin and weak stems that are easy to incline and break, resulting in stem lodging at the full flowering stage [3]. To improve the ornamental value of potted plants, plant growth regulators are one of the most economical, convenient and rapid means [4-6]. In our previous study it was found that, compared with paclobutrazol and chlormequat, the application of uniconazole to the leaves of *P. lactiflora* 'Dafugui' most improved its ornamental value by producing a dwarfing effect with stronger stems and greener leaves without negative effects on flowering [7]. However, the effects of uniconazole on the physiological and biochemical characteristics of *P. lactiflora* are unknown.

Uniconazole ((E)-(RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl) pent-1-en-3-ol), or S-3307, is a plant growth retardant that belongs to a group of triazoles [8-9]. Uniconazole can alter the endogenous levels of hormones including gibberellic acid (GA_3), indole-3-acetic acid (IAA), *trans*-zeatin riboside (ZR) and abscisic acid (ABA), and inhibits gibberellins biosynthesis [9-10]. The biosynthesis of gibberellins consists of three steps: firstly, copalyl pyrophosphate synthase and *ent*-kaurene synthase convert geranylgeranyl diphosphate to *ent*-kaurene; secondly, *ent*-kaurene oxidase converts *ent*-kaurene to *ent*-kaurenoic acid, which is converted further by *ent*-kaurenoic acid oxidase to produce GA_{12}; finally, GA_{20}oxidase and GA_3oxidase change GA_{12} to bioactive GA_1, GA_3, GA_4 and GA_7 [11]. Uniconazole, which functions between the first and second steps, can inhibit the activity of *ent*-kaurene oxidase and alter the content of endogenous hormones in plants [12-13]. Uniconazole at 10 mM almost completely inhibited the activity of ABA 8'-hydroxylase in the ABA catabolism pathway in *Arabidopsis* [8]. Uniconazole was also an inhibitor of cytochrome P450 monooxygenase CYP735 and inhibited *trans*-zeatin biosynthesis in *Arabidopsis* [14]. Other studies showed that uniconazole significantly improved the ZR content in maize (*Zea mays* L.) and changed the endogenous levels of ABA and IAA in soya bean (*Glycine max* L.) [10, 15].

Uniconazole can delay plant growth, increase photosynthetic pigment content, enhance stress tolerance and improve the yield of plants [8-9, 16]. As a plant growth retardant, it has a higher activity than paclobutrazol, displays high efficiency and low toxicity, and gives small soil residue [17]. Its effects on crop growth and development, stress resistance, and physiological and biochemical characteristics, among others have been studied in rice [18], wheat [19] and other plants [20-21]. There are also similar studies on ornamental plants such as petunia (*Petunia hybrida*) [22] and sea marigold (*Borrichia frutescens*) [23], but no reports yet on the effects of uniconazole on herbaceous peony (*Paeonia lactiflora* Pall.). In this study the antioxidant defense system and metabolite and endogenous hormone contents in *P. Lactiflora* when treated with uniconazole, which has been poorly examined, are investigated.

MATERIALS AND METHODS

Experimental Materials

Paeonia lactiflora 'Dafugui' (4 years old) was purchased from Heze City, Shandong province. Each plant contained about 8-10 plumps and robust flower buds. The root system of the plant was complete, robust, without diseases and insect pests, and free of mechanical damage.

Uniconazole (5% wettable powder, Jiangsu Sword Agrochemical Co., China) was applied to foliage at 0 (control, CK: clean tap water), 20 (U20), 40 (U40) and 60 (U60) mg·L^{-1}

Experimental Design

The experiment was conducted in the greenhouse of the National Engineering Research Center for Floriculture (Xiaotangshan Town, Changping district, Beijing, China; 40°17′N, 116°39′E). Based on a preliminary experiment, the cultivation and management techniques of potted *P. lactiflora* were mainly those of Qin [1] and Yu [24], with slight improvements. Uniconazole was dissolved in water to make the required concentration; then Tween-20 (Biorigin Inc., China) was added to make up 0.17%. Tween-20 enhances adsorption by the leaf surface. When leaves began to unfold, uniconazole (U20, U40 and U60) or CK was applied as a foliar spray to the abaxial and adaxial surfaces using an 8-L pressure aerosol-propelled sprayer (Taizhou Deepbang Horticultural Products Co., China). Plants were sprayed once a week for four weeks. Each pot plant was evenly sprayed with 250 mL of each uniconazole concentration each time. Four developmental stages of *P. lactiflora* were identified: leaf-expansion stage (S1), flower-bud stage (S2), pigmented stage (S3) and full-flowering stage (S4) [25]. Healthy and uniform-sized plants were randomly selected. Leaves in the middle of the plant were used as samples, which were wrapped in tin foil and immediately frozen in liquid nitrogen, then taken back to the laboratory and stored at –80ºC for determination of physiological and biochemical characteristics.

Resistance Indices

The nitro-blue tetrazolium method was used to determine superoxide dismutase (SOD, EC 1.15.1.1) activity [26]. Guaiacol colorimetry was used to determine peroxidase (POD, EC 1.11.1.7) activity [26, 27]. Ultraviolet colorimetry was used to determine catalase (CAT, EC 1.11.1.6) activity [27, 28]. The thiobarbituric acid method was used to determine malondialdehyde (MDA) content [26].

Metabolite Content

Soluble protein content was determined using Coomassie brilliant blue G-250 staining [29]. The anthrone method was used to determine soluble sugar content [26]. The 3,5-dinitrosalicylic acid method was used to determine sucrose and reducing sugar contents [28].

Endogenous Hormones

The methods of extracting, purifying and determining ABA, IAA, ZR and GA_3 were as described by Yang et al. [30]. Samples (0.5g) were ground in 10 mL 80% (v/v) methanol (containing 1 mM butylated hydroxytoluene as antioxidant) in an ice-cooled mortar. The extract was incubated at 4°C for 4 hr, and then centrifuged at 3500 rpm for 8 min. also at 4°C. The supernatant was passed through Chromosep C18 column (C18 Sep-Pak cartridge, Waters Corp., USA) that had been prewashed with 1 mL of 80% (v/v) methanol, then 5 mL of 100% methanol. Eluted hormone fractions were washed with 5 mL ether and 5 mL methanol, then dried down by pure N_2 and dissolved in 2 mL phosphate-buffer saline containing 0.1% (v/v) Tween-20 and 0.1% (w/v) gelatin (pH7.5) to quantify free ABA, IAA, ZR and GA_3 by indirect competitive enzyme-linked immunosorbent assay (icELISA). The protocols for immunogen, coating antigen, immunisation, antisera collection, cell fusion, antibody production and purification, and monoclonal antibody (mAb) epitope determination were provided by Prof. Baomin Wang of China Agricultural University (Beijing, China) and performed as described previously [30-32]. The procedure of icELISA was carried out in Prof. Zhongpei He's laboratory (China Agricultural University, Beijing, China) and the ZR, IAA, GA_3 and ABA contents were calculated as described by Weiler et al. [32].

Statistical Analysis

All data represent the average of at least three replicates with standard deviations. Experimental data were analysed using one-way ANOVA and significance differences between means were tested with the least significance difference (LSD) test at $P < 0.05$. Statistical analyses were performed using SPSS version 25.0 (SPSS Inc., USA) and Excel 2011.

RESULTS AND DISCUSSION

Stress Resistance Indices

The activities of SOD, POD and CAT in the leaves of potted *P. lactiflora* generally show an initial rising trend in the early stages of plant growth and development (S1 to S3), peaking at S3 and falling at S4 (Figure 1). Across all four stages, SOD, POD and CAT activities are significantly highest in the U40 treatment in S3. However, there is no clear trend in MDA content among uniconazole treatments and the control in all growth stages.

Uniconazole can influence the activities of antioxidant enzymes [33], change plant morphology and cause a series of physiological response [33]. SOD, POD and CAT are closely related to the growth and development of plants. Their synergistic actions can lead to the removal of free radicals produced by membrane lipid peroxidation, reduce the production of MDA, maintain a low level of reactive oxygen species (ROS) in cells, reduce the damage to cell membranes and improve the stress resistance of plants [35].

In *Magnolia wufengensis*, compared with the control, five spraying applications of 1500 $mg{\cdot}L^{-1}$ uniconazole resulted in the best dwarfing effect, increased SOD and POD activities, increased soluble protein and soluble sugar contents, and reduced MDA content in leaves, thereby enhancing the stress resistance of the plant [36]. Yang et al. [19] found that 20 $mg{\cdot}kg^{-1}$ uniconazole dry seed dressing increased SOD, POD and CAT activities in wheat seedlings, reduced MDA content, reduced electrolyte permeability, and increased the free proline content of metabolites, thereby improving stress resistance. Zheng et al. [37] found that foliar application with 0.1 $mmol{\cdot}L^{-1}$ uniconazole significantly improved the antioxidant system (by increasing SOD, APX and POD activities and reducing cell membrane permeability and MDA) in *Kandelia obovata* leaves under low temperature stress, thus alleviating freezing-induced cell damage. Zhou [38], treating *Chlorophytum capense* at a certain concentration range and various application times, demonstrated that uniconazole could enhance antioxidant enzyme activities (including CAT, SOD and POD), reduce MDA content, and increase soluble protein and chlorophyll contents, thereby enhancing the stress resistance of *C. capense*. In our study 40 $mg{\cdot}L^{-1}$ uniconazole compared with the control increases SOD, POD and CAT activities, indicating that uniconazole might improve the resistance of *P. lactiflora* in adverse conditions.

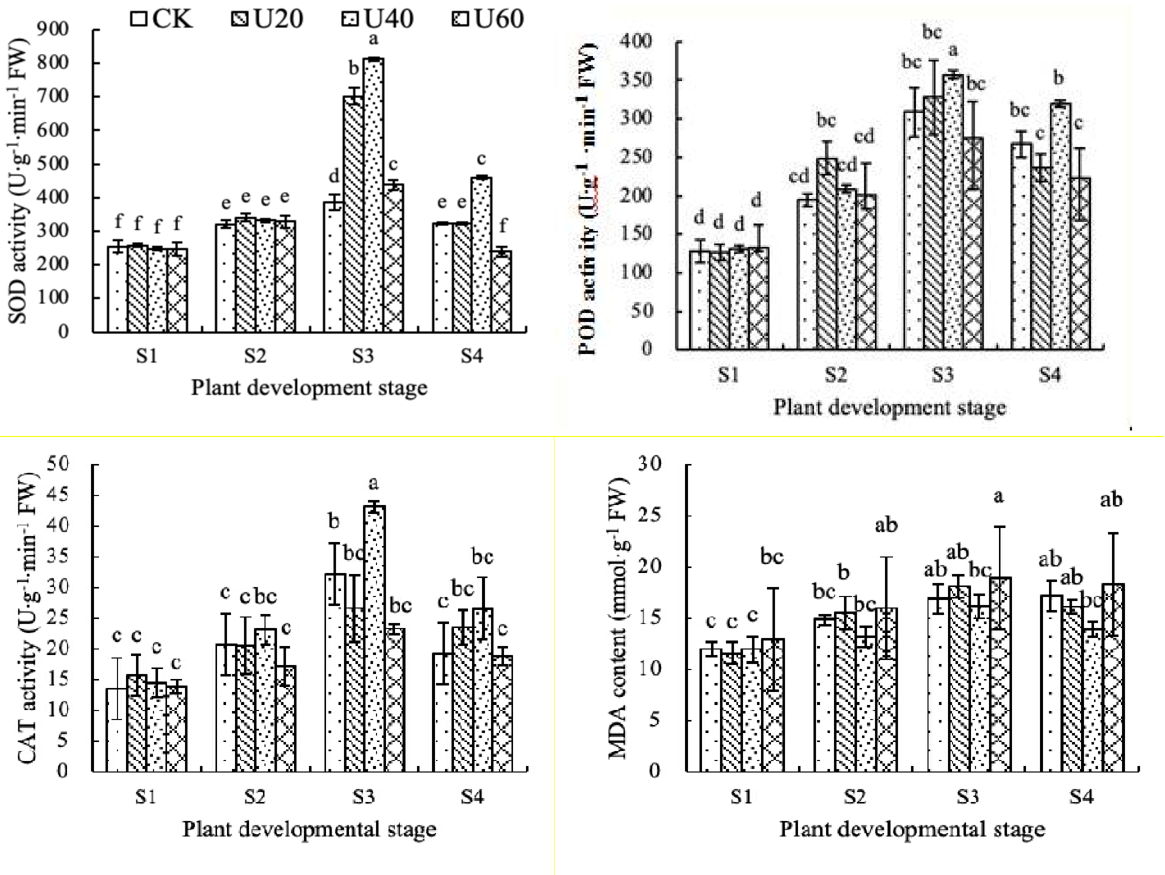

Figure 1. Effects of uniconazole treatment (U20, U40, U60) on SOD, POD and CAT activities and MDA content respectively in leaves of potted *P. lactiflora* at four developmental stages. (FW = fresh weight; vertical bars indicate means ± SD (n=3); different lower-case letters indicate significant differences across all treatments ($P < 0.05$; LSD test).)

Metabolite Content

From the results shown in Figure 2, soluble protein content is significantly highest in the U40 treatment at all growth stages, while soluble sugar, sucrose and reducing sugar contents are significantly highest in the U40 treatment at the S4 stage. Although the reducing sugar content is not as clear as those of the other metabolites, significantly highest content is observed in the U40 treatment at the S3 and S4 stages.

Soluble protein and sugar have important effects on plant physiology and are closely related to plant stress resistance [39]. Soluble protein is related to many physiological and biochemical reactions of plants. It not only is an important osmoregulator and nutrient in plants, but is also a basic unit for the synthesis of some important proteins. Since the increase and accumulation of soluble protein can improve the retaining water ability of cells and protect their biofilms, it is often used as an index for screening resistance [40]. When plants experience stress, they adapt by increasing soluble protein content [41]. On the other hand, a large amount of sugar in plant cells reduces water potential, increases water retaining ability, and lowers its freezing point [42]. Increasing the contents of soluble sugar, sucrose and reducing sugar can improve the resistance of plants to abiotic stress [43]. These four physiological indicators (soluble protein, soluble sugar, sucrose and reducing sugar) might jointly explain the ability of *P. lactiflora* to cope with adverse environmental conditions.

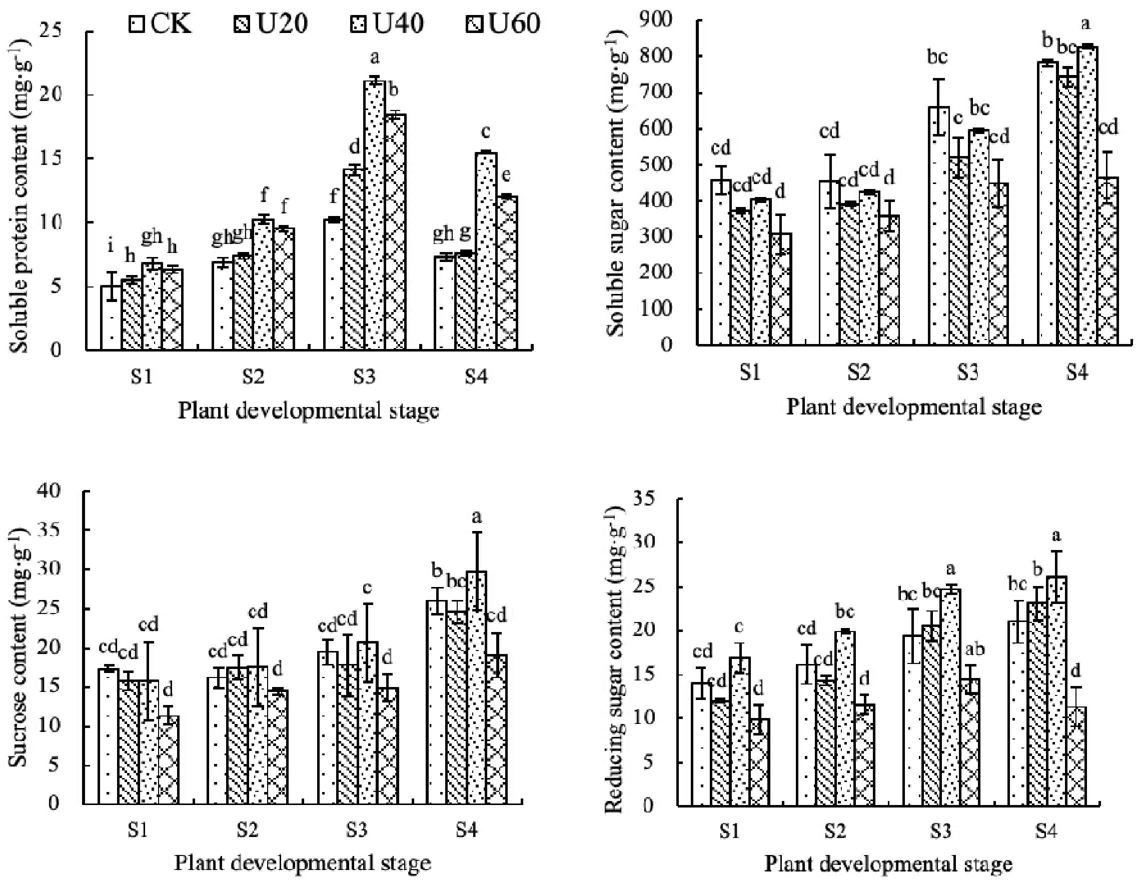

Figure 2. Effects of uniconazole treatment (U20, U40, U60) on soluble protein, soluble sugar, sucrose and reducing sugar contents respectively in leaves of potted *P. lactiflora* at four developmental stages. (vertical bars indicate means ± SD (n=3); different lower-case letters indicate significant differences across all treatments ($P < 0.05$; LSD test).)

Xiang et al. [44] found that 20 mg·L^{-1} uniconazole alleviated low temperature injury of *Vigna angularis* and reduced the effect of low temperature on the bean yield by increasing the content of soluble sugar, soluble protein and proline and the activities of protective enzymes (SOD, POD, CAT), and decreasing MDA content. Li et al. [22] used 30 mg·L^{-1} uniconazole to treat *Petunia hybrida* (irrigated with solutions containing 200 mmol·L^{-1} NaCl) and found that the treatment reduced the salt injury of seedlings and increased soluble sugar, proline and soluble protein contents, which improved salt resistance. In our study soluble protein content was significantly highest in the U40 treatment in all four growth stages while soluble sugar, sucrose and reducing sugar contents were significantly highest in the U40 treatment at the full-flowering stage, suggesting that the application of 40 mg·L^{-1} uniconazole to potted *P. lactiflora* might improve their stress resistance.

Endogenous Hormones

From the results shown in Figure 3, the U20 treatment significantly gives rise to the highest content of IAA and GA_3 at the S4 stage and the control of S4 stage respectively. Significantly highest ZR content is broadly observed in the U20 and U40 treatments of S2, the U40 and U60 treatments of S3, and the control of S4, while significantly highest ABA content is observed in the U60 treatments of S2 and S4.

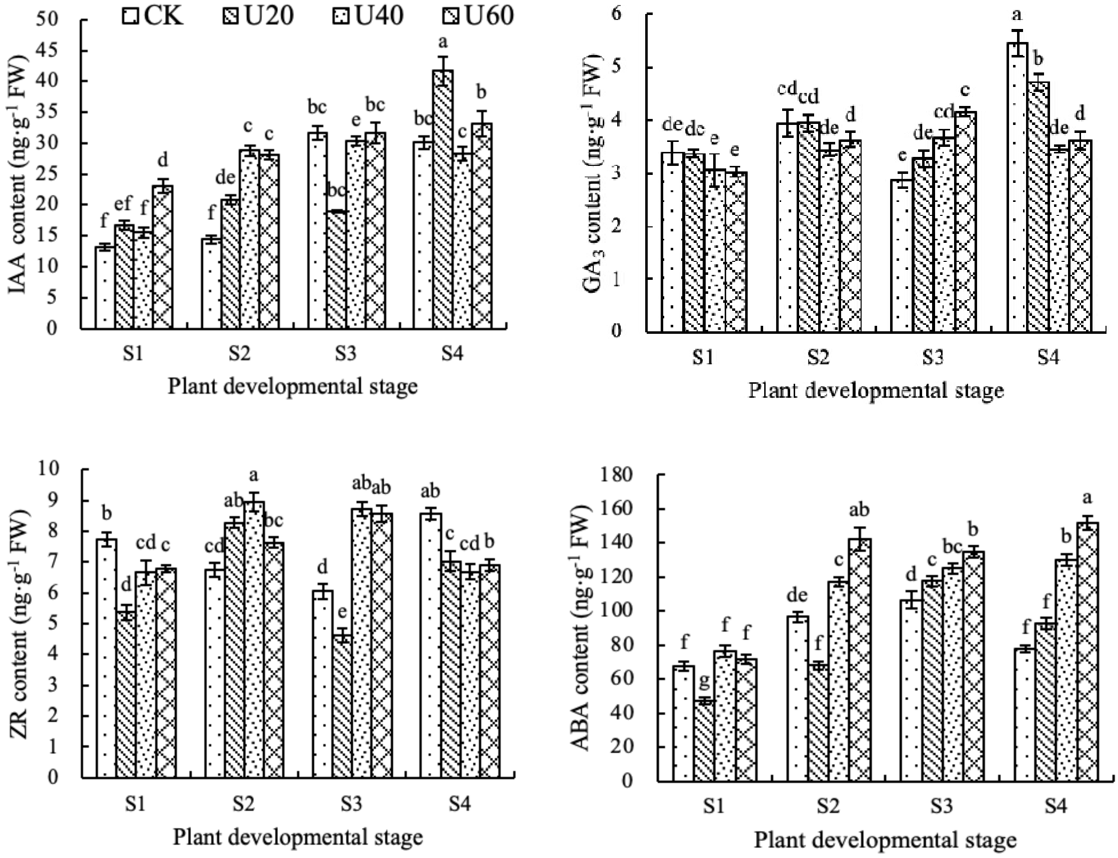

Figure 3. Effects of uniconazole treatment (U20, U40, U60) on contents of endogenous hormones IAA, GA_3, ZR and ABA respectively in leaves of potted *P. lactiflora* at four developmental stages. (FW = fresh weight; vertical bars indicate means ± SD (n=3); different lower-case letters indicate significant differences across all treatments ($P < 0.05$; LSD test).)

Endogenous hormones regulate the growth and development of plants through complex signal transduction pathways, thereby exhibiting network feedback regulation that controls cell differentiation and proliferation [45]. ABA can affect the contents of SOD, POD, MDA, soluble protein and soluble sugar, and reduce damage caused by drought or low-temperature stress [46].

Many studies showed that uniconazole foliar spray treatment reduces the level of IAA, GA_3 and ZR in plants, while the level of endogenous ABA increases [10, 16, 47]. Bakheta and Hussein [48] sprayed different concentrations of uniconazole (150, 200 $mg \cdot L^{-1}$) to *Hordium vulgare* under salt stress (2500, 5000 $mmol \cdot L^{-1}$), resulting in an accumulation of ABA, cytokinin, crude protein, total soluble protein, proline, IAA and GA_3. Li and Liu [49] treated *Lilium Asiatic* hybrids with uniconazole and found that it could significantly reduce the content of IAA and GA_3 and increase the content of zeatin riboside (ZR) and ABA. In our study the U40 treatment at the full-flowering stage decreased the content of IAA, GA_3 and ZR in *P. lactiflora* leaves by 5.78%, 4.82% and 22.03% respectively, but increased the ABA content by 67.58%.

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CONCLUSIONS

The effects of uniconazole treatment on *P. lactiflora* are likely very complex. Stress resistance indices, metabolite content and endogenous hormones are important physiological and biochemical characteristics of plants. Compared with control, the optimal treatment for improving *P. lactiflora* adaptability to the environment is spraying uniconazole at 40 mg·L^{-1}. This treatment not only improves the ornamental value of the potted plant, but also results in altering the main biochemical and physiological parameters directly linked to stress resistance to improve its adaptability to the environment. It is still necessary to further explore the regulating mechanisms of uniconazole by molecular means in the future to provide more powerful guidance for uniconazole application in ornamental plants.

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