Effect of paclobutrazol on *Narcissus tazetta*: endogenous cues to improve flowering

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Abstract

Paclobutrazol (PBZ) retards plant growth through reducing gibberellin biosynthesis. Flowering is critical for *Narcissus tazetta* as a unique cut flower with commercial value. Hence, improving its quality and yield is crucial in the cultivation of *N. tazetta*. This study was carried out to investigate the effect of PBZ treatment (25, (50 and 100 mM) on physiological, morphological and anatomical characteristics in *N. tazetta*. Pre-treatment of *Narcissus* bulbs with different PBZ levels induced a significant increase in the flowering percent, flower size, scape diameter, phloem and xylem vessels, pith cavity size, leaf thickness, spongy and palisade mesophylls, and vascular bundle width, despite a significant decrease in height and fresh mass of scape at 100 mM. Mean germination time of bulbs and flowers significantly increased at 50 and 100 mM PBZ while germination index of bulbs showed a significant increase at 25 mM. Under PBZ treatment, the observed increase in the photosynthetic pigments was followed by high accumulation of sugars. Moreover, different concentrations of PBZ significantly increased anthocyanin, phenols, proteins, proline, and total antioxidant activity in the *Narcissus* leaves and flowers. No changes in H_2O_2, malondialdehyde, and activity of catalase and ascorbate peroxidase can be related to no stress-inducing effects of PBZ treatment in *Narcissus*. Overall, the positive effect of PBZ on physiological, morphological, and anatomical function in flowering procedure in *N. tazetta* offers suggestions of its future usage in other ornamental bulbous plants.

Keywords: germination, morphological parameters, physiological characteristics, plant anatomy, plant growth retardant

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Introduction

Floral induction is critical for ornamentals to meet commercial demands (Chandel et al., 2023; Mekapogu et al., 2022). Numerous studies have been conducted to establish the physiological mechanisms behind flowering process and factors improving the quality and longevity of cut flowers (Gun et al., 2023; In and Lim, 2018; Kaviani et al., 2023). Recently, researchers have paid high attention to the endogenous physiological and molecular mechanisms related to floral regulation (Chen et al., 2023; Fan et al., 2018). The interactions between endogenous and environmental cues regulate the plant growth, switching from vegetative to flowering phase (Fan et al., 2018). Six important pathways have been

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introduced to play roles in controlling flowering, including photoperiod, vernalization, ambient temperature, autonomous, gibberellin, and age (Kumar et al., 2023; Silverstone et al., 2007).

Gibberellin (GA) is one of the widely-studied endogenous phytohormones in plants, which involves in regulating flowering process in plant species (Skalicky et al., 2020). For example, GA treatment accelerated *Arabidopsis* flowering, particularly under short days. A disruption in either GA biosynthesis or signaling induced changes in flowering time (Moon et al., 2003; Xu et al., 2022). The inhibition of GA biosynthesis has been considered as a possible primary mechanism restricting the vegetative growth and promoting flowering (Upreti et al., 2013; Wei et al., 2021). The *Arabidopsis* mutant *ga1-3*, with a deletion in the gene encoding ent-kaurene synthetase, fails to bloom in short day conditions, which reveals the necessity of applying GA in flowering induction in short-day conditions (Moon et al., 2003; Ritonga et al., 2023). Differently, the mutant *spindly*, with the active GA signaling, blooms under both short- and long-day conditions (Jacobsen et al., 1996; Silverstone et al., 2007). Hence, the application of a GA-inhibitor compound, which alters flowering process, has been proposed in the present study.

Growth retardants are a group of synthetic plant growth inhibitors regulating the plant growth through retarding cell division and preventing shoot elongation (Qian et al., 2022). Paclobutrazol (PBZ) from triazole family have been used since 1985 in cultivation of plants with various purposes, including increasing quality and size of fruits, improving strength of plants against lodging, inducing flowering, and enhancing plant tolerance to environmental stresses (Usenko et al., 2023). PBZ retards GA biosynthesis process due to suppression of ent-kaurene oxidase activity in the ent-kaurene oxidation pathway (Nagar et al., 2021; Wei et al., 2021). PBZ abolishes plant growth based on the plant species, developmental stage, and morphological, anatomical, and physiological features, as well as its concentration and method of application. It can induce modifications in mesophyll cells, leaf area and thickness, stem diameter, and size of vascular bundle (Oliveira et al., 2020; Tsegaw et al., 2005). PBZ can induce leaves with darker green color due to an increase in the chlorophylls contents (Desta and Amare, 2021; Sharma et al., 2023).

Narcissus tazetta is a perennial ornamental plant from the family Amaryllidacese containing bulb. *Narcissus* has 3-20 fragrant flowers in a scape, which is widely used in the medical and cosmetics industries due to its alkaloid compounds. *N. tazetta* with flat flower cover and hemispherical flower crown is one of the most favorable species of *Narcissus* genus. Locally called "PorPar" *N. tazetta* in Iran refers to a *Narcissus* flower with double petals known as "Double Roman" (Gholami et al., 2018; Hajihashemi and Jahantigh, 2022). As a winter-flowering plant in the Mediterranean area, it blooms at temperatures ranging between 10-15 ℃ and 3-10 ℃ during the day and night, respectively (Terry et al., 2021). The southern parts of Iran, including Khuzestan, due to the mild winters, are important habitats to propagate ornamental plants such as *Narcissus* species (Daneshvar and Heidari, 2011). Behbahan Khuzestan, Iran, is known as one of the most famous habitats of *N. tazetta*, with a suitable market for cut *Narcissus* flower in winter (Gholami et al., 2018; Hajihashemi and Jahantigh, 2022).

With respect to high ornamental and commercial values of *N. tazetta* and reported positive effect of PBZ on flowering process (Demir and Çelikel, 2018; Upreti et al., 2013; Zhang et al., 2016), the present study was designed to investigate the effect of PBZ treatment on physiological, morphological, and anatomical characteristics of *N. tazetta*. In this regard, the effect of PBZ on flowering process was studied, along with, postharvest quality of cut flowers, photosynthetic and non-photosynthetic pigments, metabolites, and antioxidants.

Materials and Methods

N. tazetta bulbs were purchased from Narges Zar PA, Behbahan, Khuzestan, Iran. The study was performed under field conditions from November 2019 to February 2020 in Behbahan. The bulbs were pre-treated with four levels of PBZ, including 0, 25, 50, and 100 mM to determine the best concentration for floral induction. Forty-eight uniform bulbs (30-40 g) were selected with twelve

Narcissus bulbs per each pretreatment incubated in 0 (distilled water), 25, 50, and 100 mM PBZ, and kept in a dark place for 24 hours. The bulbs were cultivated in rows with fifty centimeters distance and at a depth of twice height of the bulbs on 11th of November 2019. The soil pH was about 6.5, containing about 2-4% organic matter and an electrical conductivity of 0.18 dS m^{-1}. Based on the analysis of Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) (Baker and Amacher, 1983; Rashed, 2010), the soil contained Ca at 0.45% , Cu at 14 mg Kg^{-1}, Fe at 82 mg Kg^{-1}, K at 1.18% , Mg at 0.16%, Mn at 25 mg Kg^{-1}, Na at 0.04%, P at 0.25%, S at 0.11%, and Zn 17 mg kg^{-1}. The soil nitrogen content based on the micro-Kjeldahl analysis (Bremner, 1960) was about 1.34%. In November, the air temperature during days ranged between 27 and 14 ℃. The bulbs were irrigated weekly with tap water at eighty percent field capacity, based on the method of soil weighing (Hajihashemi, 2020). The emergence and development of aerial parts of plants were documented until the final stage of flowering process. The first emergence of leaves and flowers were recorded on 23 of November and 24 of December, respectively. The flowering season continued until 2nd of February. Then, the flower and leaf samples were harvested for further morphological, physiological, and anatomical analysis as explained in following sections.

Morphological, vase life, and physiological and anatomical analyses

The observations of developmental process of *Narcissus* at each treatment were recorded daily to calculate the germination index (GI) and mean germination time (MGT) of bulbs, flower buds, and fully extended flowers. The data of first day of emergence of different organs, including green young leaves from soil, flower buds, and fully extended flowers until the last day of their occurrence per each treatment were used to calculate their MGT and GI indexes using the following equations:

MGT = Σ (f × i) / Σ f (Ellis et al. 1980), where f is the number of newly emerged organs on day i.

GI = Σ (Gi / Ti) (Wang et al. 2004), Gi = percentage of emerged organs at ith day, and Ti = day of emergence.

The number of blooms per scape were recorded after blooming the maximum buds per each plant. Immediately after harvesting, the height of plants, flowers diameter, and fresh weight of flowers were measured. Then, flowers were dried at 70 ℃ for 48 hours and their dry weight were measured.

To study on the vase life of cut flowers, three flowering stalks with height of 30 cm at each treatment were cut and immediately transferred to containers filled with distilled water. Vase life was monitored daily based on the emergence of symptoms such as petal folding and flower wilting, which led to loss of flowers attractiveness and marketability (Hajihashemi and Jahantigh, 2022; Kaviani et al., 2023).

Photosynthetic pigment contents were measured in fresh leaves based on Wellburn (1994) assay. The fresh leaves and flowers were used to assay water soluble carbohydrates (WSC) according to the method of DuBois et al. (1956). Soluble proteins were extracted from fresh leaves and flowers using sodium phosphate buffer and measured by Bradford (1976). The activity of antioxidant enzymes were measured according to the procedures developed for catalase (CAT) (EC 1.11.1.6) (Aebi, 1984) and ascorbate peroxidase (APX) (EC 1.11.1.11) (Nakano and Asada, 1987). Fresh leaves and flowers were homogenized in sulfosalicylic acid solution and ninhydrin reagent was used to analyze proline content (Bates et al., 1973).

Phenol contents of fresh samples were measured using Folin's reagent (Singleton and Rossi, 1965). Anthocyanin contents of fresh leaves and flowers were measured according to Wagner (1979) method. Measurement of H_2O_2 in the fresh leaves and flowers was done based on the method of Velikova et al. (2000). Thiobarbituric acid assay was used to measure the malondialdehyde (MDA) contents of fresh leaves and flowers (Heath and Packer, 1968). Total antioxidant activity (adaptation of FRAP) in fresh leaves and flowers

was measured according to Szôllôsiand Varga (2002). The hand-cut of leaves and scape (flowering stalk) were stained in toluidine blue O (0.02%), and observed and photographed by a light microscope (Persson et al., 2005). Anatomical features were assessed using Image Tools software Version 3.0 (Hajihashemi and Jahantigh, 2022).

Statistical Analyses

The bulbs were planted in four detached plots per treatments. After harvesting the plants, every organ of three *Narcissus* plants per each plot were mixed completely and considered as one replication for each treatment. The statistical analysis was done based on the one-way analysis of variance (ANOVA) using SPSS (version 24). The Tukey's test (P≤0.05) was applied to determine the significant differences among treatments. The average of three independent biological replicates were reported.

Results

Based on the analysis of variance (ANOVA), PBZ induced statistically significant changes in the measured vegetative and flowering traits in *Narcissus*. The GI of bulbs significantly increased in response to 25 mM PBZ (55%) while no significant differences were achieved at 50 and 100 mM PBZ (Fig. I. A). Except for 25 mM PBZ, 50 and 100 mM PBZ increased MGT of bulbs by 8% and 7%, respectively, higher than in the control plants (Fig. I. B). MGT of flower buds and mean of flowering time exhibited a significant increase at 50 and 100 mM PBZ while they showed insignificant differences at 25 mM PBZ (Figs. I. C & D). PBZ concentrations of 25, 50, and 100 mM increased the flowering percentage by about 20%, 20%, and 33%, respectively, greater than in the control plants (Fig. I. E). Moreover, the number of flowers per scape significantly enhanced at 25 and 50 mM PBZ, whereas no significant changes were recorded at 100 mM PBZ (Fig. I. F).

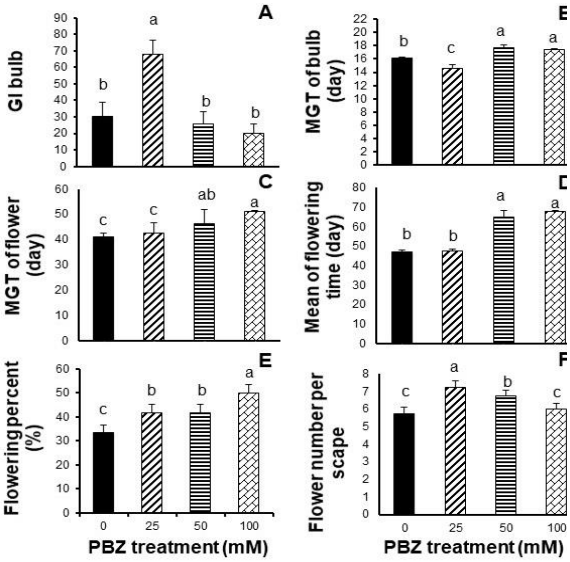

Fig. I. (A) Germination index of bulb, (B) mean germination time (MGT) of bulb, (C) mean germination time (MGT) of flower bud, (D) mean flowering time, (E) flowering percent, and (F) number of flowers per scape in *Narcissus tazetta*-treated with different concentrations of paclobutrazol (0, 25, 50, and 100 mM); the same lower-case letters on columns show no significant difference at P≤0.05.

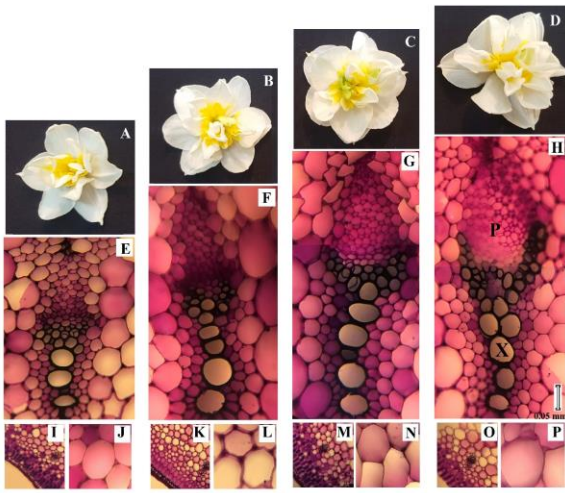

Fig. II. *Narcissus tazetta* flowers under different levels of PBZ; A: control, B: 25 mM, C: 50 mM, and D: 100 mM; cross section of scale vascular tissues at E: 0 mM, F: 25 mM, G: 50 mM, and H: 100 mM PBZ; micrograph of flower scale cross section, I and J: 0 mM PBZ (×100 and ×400 magnifications, respectively); K and L: 25 mM PBZ (×100 and ×400, respectively); M and N: 50 mM PBZ (×100 and ×400, respectively), and O and P: 100 mM PBZ (×100 and ×400 magnifications, respectively); P: phloem vessel and X: xylem vessel.

The comparison of means showed that all concentrations of PBZ increased the flowers size (Figs. II. A-D & III. A). The largest (50.4 mm) and smallest (45.2 mm) flower diameters were obtained at 100 and 25 mM PBZ, respectively. In addition, various PBZ treatments enhanced the flowers' fresh and dry mass, with the greatest increase being related to 100 mM (Figs. III. B & C). The concentration of 100 mM PBZ significantly decreased the height of flowers scape while 25

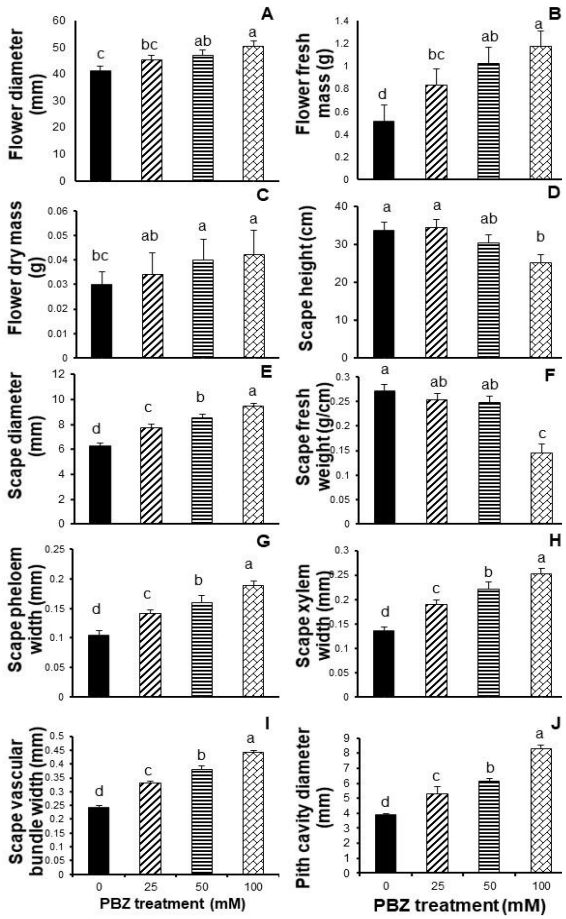

Fig. III. *Narcissus tazetta* treated with different concentrations of paclobutrazol (0, 25, 50 and 100 mM); (A) flower diameter, (B) flower fresh mass, (C) flower dry mass, (D) scape height, (E) scape diameter, (F) scape fresh mass, (G) scape phloem width, (H) scape xylem width, (I) scape vascular bundle width, and (J) pith cavity width in; the same lower-case letters on columns show no significant difference at P≤0.05.

mM and 50 mM PBZ did not significantly differ from the control plants (Fig. III. D). All PBZ concentrations significantly improved the scape diameter, with the maximum increase (34%) being related to 100 mM (Fig. III. D).

According to Fig. (III), the observed increase in the scape diameter was partly due to more vessels production. The concentrations of 25, 50, and 100 mM PBZ significantly increased the width of scape phloem and xylem vessels by 26%, 35%, and 44% and also by 28%, 38%, and 46%, respectively, greater than those in the control plants (Figs. III. G & H). Along with the improvement in width of vessels, PBZ treatment also increased the width of

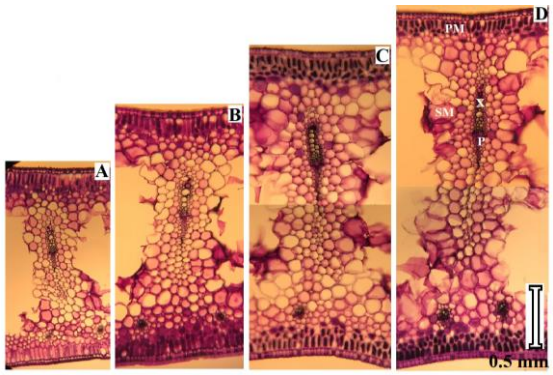

Fig. IV. Micrograph of leaf cross section of Narcissus tazetta flowers treated with different concentrations of paclobutrazol: (A) control, (B) 25 mM, (C) 50 mM, and (D) 100 mM; P: phloem vessel, PM: pallisade mesophyll, SM: spongy mesophyll, X: xylem vessel.

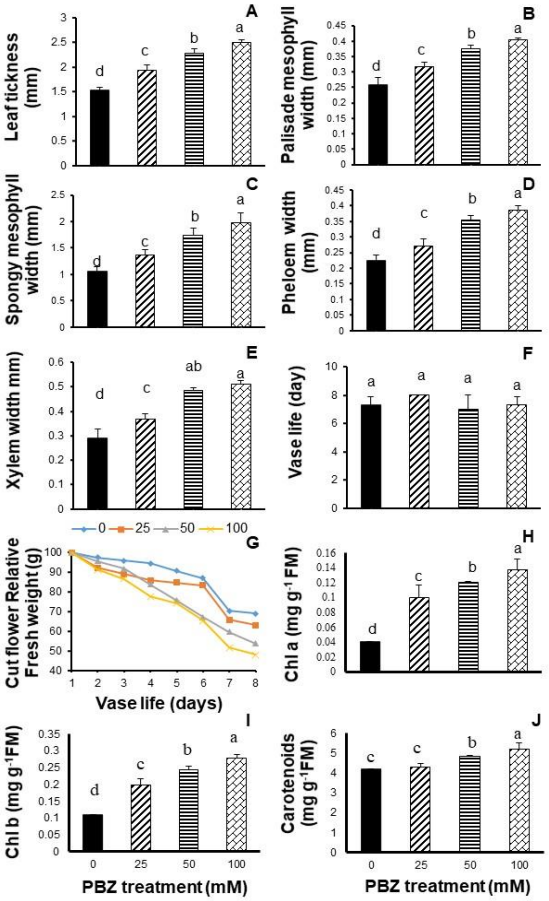

Fig. V. Narcissus *tazetta* treated with different concentrations of paclobutrazol (0, 25, 50 and 100 mM): (A) leaf thickness, (B) palisade mesophyll width, (C) spongy mesophyll width, (D) leaf phloem width, (E) leaf xylem width, (F) vase life, (G) cut flower relative fresh weight flower, (H) chlorophyll *a* (Chl *a*), (I) Chl *b*, and (J) carotenoids; the same lower-case letters on columns show no significant difference at P≤0.05.

scape vascular bundle as presented in Fig. (II. E-H) and (III. I) The comparison of means showed that the pith cavity size significantly increased at 25 (27%), 50 (36%) and 100 (53%) mM PBZ, greater than the control plants (Figs. II. I-P & III. J).

The cross-section of leaves showed that PBZ-treated plants produced thicker leaves than the control plants (Figs. IV A-D & V. A). In response to 25, 50, and 100 mM PBZ, the leaf thicknesses increased by about 21%, 33%, and 39%, respectively, thicker than the control plants (Fig. V. A). Under different concentrations of PBZ, the observed increase in the leaves thickness was due to the enlargement of palisade and spongy mesophylls, and vessels width (Figs. IV. A-D and V. B to E). The Maximum (36%) and minimum (19%) increases in the width of palisade mesophyll were observed at 100 and 25 mM PBZ, respectively (Fig. V. B). The width of spongy mesophyll exhibited a significant increase in response to 25 (22%), 50 (39%), and 100 mM PBZ (47%), comparing to the control plants (Figs. V. B & C). Under PBZ treatment, the width of phloem and xylem vessels showed significant differences from one another (Figs. V. D & E). According to the comparison of means, the maximum width of phloem (42%) and xylem (43%) vessels were related to 100 mM PBZ (Figs. V. D & E). Among PBZ-treated plants, the minimum width of phloem (17%) and xylem (21%) vessels were obtained at 25 mM (Figs. V. D and E). The vase life of cut *Narcissus* flowers showed that PBZ treatment was not putative to increase flowers postharvest life (Fig. V. F). The relative fresh weight of cut flowers reduced by half of their initial weight during 8 days in both control and PBZ-treated plants (Fig. V. G).

PBZ improved the photosynthetic and non-photosynthetic pigments production in leaves as well as non-photosynthetic pigments in flowers. Different concentrations of PBZ induced a significant increase in the chlorophylls *a* and *b*, and carotenoids contents (Figs. V. H-J). Chlorophyll *a* (86%) and *b* (72%), and carotenoids (20%) contents exhibited their greatest values at 100 mM PBZ, more than those in the control plants. The accumulation of phenols and anthocyanin in the leaves and flowers differ significantly in response to the PBZ treatments (Figs. VI. A-D). The

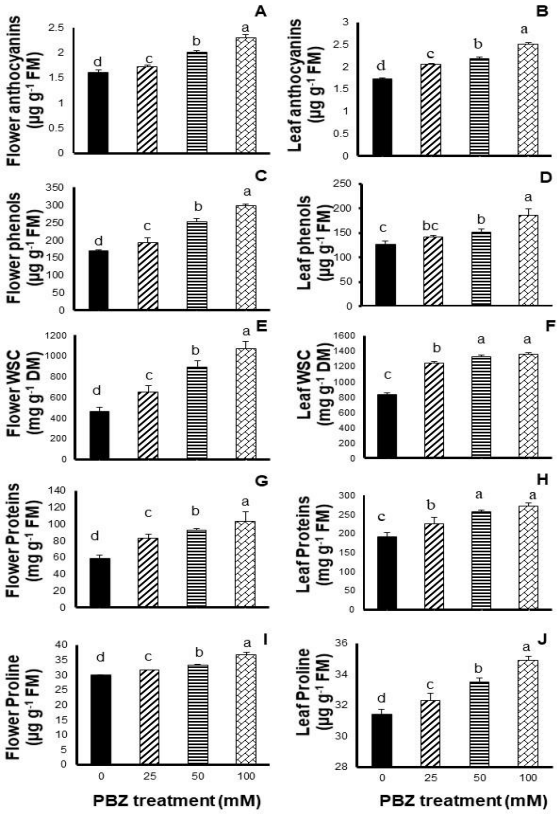

Fig. VI. *Narcissus tazetta* treated with different concentrations of paclobutrazol (0, 25, 50 and 100 mM); (A) Flower anthocyanin, (B) leaf anthocyanin, (C) flower phenols, (D) leaf phenols, (E) flower water soluble carbohydrates (WSC), (F) leaf WSC, (G) flower proteins, (H) leaf proteins, (I) flower proline, and (J) leaf proline; the same lower-case letters on columns show no significant difference at P≤0.05.

highest values of phenols and anthocyanin in the flowers and leaves were recorded with 100 mM PBZ treatment, by 43% and 30% and also 32% and 30%, respectively, greater than the control plants. The flowers accumulated greater amounts of phenols and anthocyanin than leaves. The leaves and flowers of PBZ-treated plants accumulated higher WSC contents in comparison with the control plants (Figs. VI. E and F). The highest values of WSC in leaves (39%) and flowers (56%) were related to 100 mM PBZ. Comparison of means for the data on proteins and proline revealed that their values in the PBZ-treated plants differ significantly from the control plants and one another (Figs. VI. G-J). The highest protein and proline contents in leaves and flowers were obtained at 100 mM PBZ, by 30% and 10% and also 43% and 18%, respectively, as compared with control plants (Figs. VI. G-J).

The H_2O_2 content of leaves and flowers of PBZ-treated plants did not differ from that of the control plants (Figs. VII. A and B). Moreover, the MDA contents of the flowers and leaves of PBZ-treated plants showed no significant changes, comparing to the control plants (Figs. VII. C & D). Also, the CAT activity of leaves and flowers did not change significantly in response to different concentrations of PBZ (Figs. VII. E & F). Similarly, different PBZ treatments induced no significant variations in the APX activity of leaves and flowers (Figs. VII. G & H). As well as non-photosynthetic pigments, different PBZ treatments effectively increased the total antioxidant activity of leaves and flowers as revealed with a significant increase in the FRAP value (Figs. VII. I and J). The maximum increase in FRAP value of leaves (18%) and flowers (11%) were obtained at 100 mM PBZ, compared to the control plants.

Discussion

The effect of PBZ pre-treatment on *N. tazetta* growth and development was studied in the present study. Floral induction is controlled by complex networks in response to endogenous and environmental factors. The plant hormone of GA is one of the endogenous factors regulating the floral induction (Zhang et al., 2016). Application of PBZ as a GA inhibitor has been introduced as a treatment to gain an insight into the role of GA in flowering regulation (Upreti et al., 2013; Zhang et al., 2016). Several reports suggested different effects of PBZ treatment on floral stage between different plants species, coupled with physiological process to provide potential insights into the role of GA in developmental process. Different response of floral process to PBZ between plants has received high attention. For instance, PBZ treatment was effective to induce early flowering in mango (Upreti et al., 2013) and apple (Zhang et al., 2016) while Demir and Çelikel (2018) found no changes in flowering time and number of flowers in the PBZ-treated bulbs of *N. tazetta*. In the present study, contrary to the report of Demirand and Çelikel (2018), pre-treatment of *Narcissus* bulbs with 50 and 100 mM PBZ prolonged MGT of flower, flowering time, and flowering percentage. Moreover, 25 and 50 mM

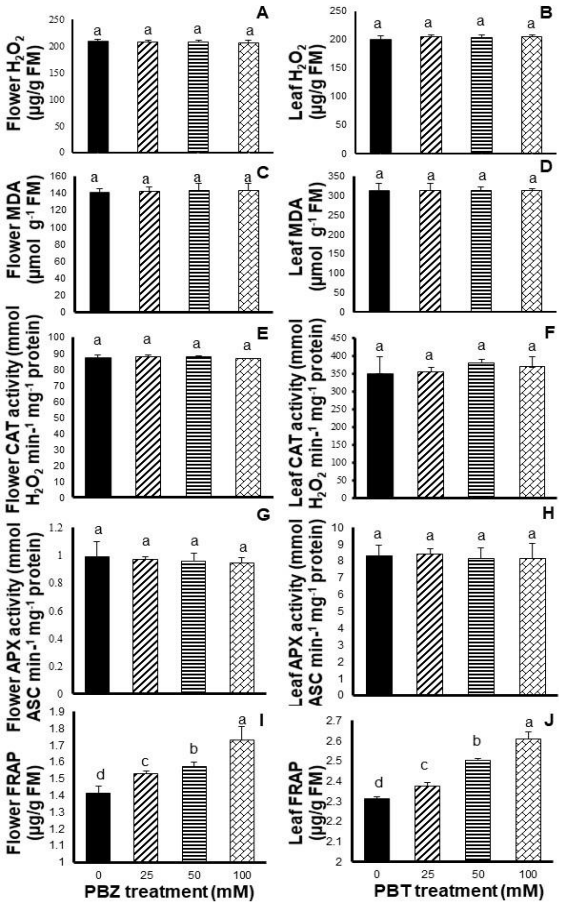

Fig. VII. *Narcissus tazetta* treated with different concentrations of paclobutrazol (0, 25, 50 and 100 mM); (A) flower H_2O_2, (B) leaf H_2O_2, (C) flower malondialdehyde (MDA), (D) leaf MDA, (E) flower catalase (CAT), (F) leaf CAT, (G) flower ascorbate peroxidase (APX), (H) leaf APX, (I) flower total antioxidant power (FRAP), and (J) leaf FRAP; the same lower-case letters on columns show no significant difference at P≤0.05.

PBZ significantly increased the flowers number, greater than the control plants. Accordingly, the responses of MGT of flower buds, mean flowering time, flowering percentage, and flowers number per scape to PBZ were dose-dependent. Similar to flowering process, the response of vegetative growth to PBZ treatment was dose-dependent. The pre-treatments of *Narcissus* bulbs with PBZ significantly increased GI at 25 mM, whereas 50 and 100 mM PBZ significantly prolonged the germination time of bulbs. Based on the obtained results, it can be suggested that high concentration of PBZ induced late vegetative and flowering growth. It has been already reported that high levels of PBZ retarded flowering process

(He et al., 2004; Khurana et al., 2011; Zhang et al., 2019), which was in line with the results of the present study. Regardless of the reported prolonged vase life of cut *Chrysanthemum* flowers by PBZ treatment (Petridou et al., 2001), the pre-treatment of bulbs with PBZ was not effective on improving postharvest life of *N. tazetta*.

Narcissus produced an average of 5 to 8 flowers per scape under different PBZ concentrations. The PBZ treatment at 25 and 50 mM maximized the number of flowers per scape while 100 mM PBZ did not increase the number of flowers. The number of *Narcissus* flowers in this study reduced at 100 and 200 mg L^{-1} PBZ pre-treatment, and comparing the findings with those of Demir and Çelikel (2018) confirms the dose-dependent effect of PBZ on the flowering process.

PBZ impedes GA biosynthesis through blocking the ent-kaurenoic acid formation in the GA biosynthesis pathway, resulting in reduction of active GAs and consequent inhibition of stem elongation (Desta and Amare, 2021; Hajihashemi et al., 2013; Wei et al., 2021). Based on the results of the present study, the PBZ treatment at 100 mM induced a significant reduction in the height of flower scape while it was not effective in 25 mM and 50 mM treatments.

In this study, PBZ treatment resulted in a significant increase in flower diameter and fresh and dry mass, which is in agreement with the previous report of Wei et al. (2021). Along with the observed improvement in the flower size, the fresh and dry mass of flowers increased in response to PBZ treatment. In order to understand the reason of observed increase in flower yield, the anatomy of plants was studied in PBZ-treated *Narcissus*. PBZ treatment increased the scape diameter, which was supported with an increase in the phloem and xylem vessels width. Besides, the observed increase in the thickness of leaves in response to PBZ treatment was due to the increase in the width of palisade mesophyll and the vascular tissues. PBZ treatment increased leaf thickness in *Catharanthus roseus* by increasing the width of cuticle, epidermis, and palisade and spongy mesophylls (Jaleel et al., 2007). Nazarudin et al. (2007) reported an increase in the leaf thickness due to increased palisade parenchyma thickness in *Syzygium campanulatum*, which approved the observed results of the present study. The increase in the vascular tissues can facilitate the transfer of minerals, nutrients, and water through the plant (Hajihashemi and Jahantigh, 2022; Savage et al., 2015). Accordingly, it can be concluded that PBZ induced bigger flowers through increasing the vascular tissues and providing higher nutrients required for the floral induction.

PBZ increased *Narcissus* scape diameter due to induction of wider vascular bundles and larger pith diameter connected with bigger pith cells as shown in Fig. (II). Tsegaw et al. (2005) reported larger pith cells in the stem of PBZ-treated potato, which confirms the results observed in the present study (Figs. II. I & P). This modification may be attributed to suppressed cell lengthening and promoted radial expansion of cells due to the reduction in endogenous GA in response to the PBZ treatment (Tsegaw et al., 2005; Wang and Lin, 1992). Endogenous GA is necessary for regulating relative longitudinal growth, and its suppression due to PBZ treatment promoted radial cell expansion rather than longitudinal growth (Wang and Lin, 1992). In contrast to the observed increase in the scape diameter in the PBZ-treated *Narcissus*, its fresh weight decreased. In line with increasing the scape diameter, the pith cavity size increased in PBZ-treated *Narcissus*. The enlargement of the pith cavity can explain the observed reduction in the scape fresh weight in response to PBZ treatment. Guo et al. (2019) reported that the pith cavity formation in bamboo is due to the programmed death of pith cells, which is critical for overcoming the bending force. So far, thicker scape and enlarged vascular bundle size, accompanied by larger pith cavity due to the death of pith cells may have been established for providing sufficient nutrition for induction of bigger flowers and for overcoming the bending force due to enlargement of flower size in PBZ-treated plants. Further study on physiological characteristics might provide a deeper understanding of the function of PBZ in plants, which is discussed below.

The results of anatomical and physiological observations exhibited a correlation among the flowers size, leaf thickness, chlorophylls content, WSC synthesis, and vascular tissues development, all of which exhibited a similar trend in response to PBZ treatment. The pre-treatment of *Narcissus* bulbs with PBZ increased the production of chlorophylls *a* and *b*, with the highest value being obtained at 100 mM. The higher chlorophyll production in PBZ-treated plants supposed to be related to the stimulation of cytokinin synthesis that increases chloroplast differentiation and chlorophylls biosynthesis, and prevents chlorophylls degradation (Jaleel et al., 2007). High chlorophyll accumulation in the leaves of PBZ-treated plants was connected with wider palisade and spongy mesophylls and thicker leaves. The increased palisade and spongy layers in response to PBZ treatment might be due to the induced cell division followed by increase in cytokinin level (Jaleel et al., 2007; Tsegaw et al., 2005). A direct relationship was achieved between the chlorophylls and WSC values in the PBZ-treated *Narcissus*. An increase in the carbohydrates as the source of energy is required for enlargement of *Narcissus* flower size. Mishraand and Yadava (2011) reported that the flower size enlargement in PBZ treatment may be due to the energy preservation as a result of vegetative growth reduction and conversion into reproductive growth. In the PBZ-treated *Narcissus*, the export of higher WSC content from the leaves (as source) to the flowers (as sink) necessitated higher development of phloem tissues in the scape and leaves, which was achieved in the present study. The observed increase in the phloem vessel width in the PBZ-treated *Narcissus* is confirmed by previous reports which also reported a decrease in the xylem layer (Jaleel et al., 2007; Nazarudin et al., 2007), contrary to the result of present study. The effect of PBZ on the induction of anatomical and physiological modifications was mediated by a change in the hormonal balance of the plant, which, in turn, induced enlargement of flower size.

PBZ treatment promoted a significant increase in the accumulation of non-photosynthetic pigments such as phenols and anthocyanin in both leaves and flowers. High accumulation of phenols and anthocyanin with antioxidant properties (Hajihashemi et al., 2020a) may have reflected in greater FRAP value in the leaves and flowers of PBZ-treated *Narcissus*. A direct relationship was detected between the amounts of non-enzymatic antioxidants (e.g., phenols, anthocyanin, and proline), and FRAP value with inhibition of MDA accumulation in the leaves and flowers of PBZ-treated *Narcissus*. According to Fletcher et al. (2000), inhibition of lipid peroxidation may be one of the mechanisms responsible for the anti-senesces effects of PBZ. The pre-treatment of *Narcissus* bulbs with PBZ has been shown to result in higher production of proline and proteins. While a clear-cut relationship between accumulation of proline with stress adaptation has been reported, a significant raising of proline in the reproductive organs may also occur in non-stressed conditions (Verbruggen and Hermans, 2008). Proline accumulation can provide the cell with enough energy to sustain rapid growth. A positive correlation between proline and protein synthesis exists, as hydroxyproline-rich glycoproteins are important structural constituents of cell wall to play a key role in the regulation of cell division and cell extension (Verbruggen and Hermans, 2008). Accordingly, it is likely that the increased proline and protein contents played a role in enlarging the scape diameter and flowers size in the PBZ-treated plants. H_2O_2 is synthesized naturally in the photosynthesis, photorespiration, and respiration process with an indispensable role in seed germination, flowering, stomatal regulation, programmed cell death, and senescence. CAT and APX are antioxidant enzymes which catalyze dismutation of H_2O_2 to H_2O (Gohari et al., 2020; Hajihashemi and Ehsanpour, 2014; Hajihashemi et al., 2020b). The PBZ treatment induced no changes in the H_2O_2 content accompanied by no changes in the CAT and APX activity in the leaves and flowers of PBZ-treated *Narcissus*. From the lack of changes in the H_2O_2 and MDA contents, it might be concluded that PBZ treatment induced no stress in *Narcissus*.

Conclusion

Application of PBZ is a short-term alternative to provide knowledge regarding improving the

flowering process in ornamental plants. Results of the present study revealed that PBZ improved flowering in *N. tazetta* through improving the morphological, anatomical, and physiological characteristics, without inducing stress in plants. Based on the results of the present study, *Narcissus* response to PBZ was dose-dependent because the shortest flower scape with the highest scape diameter along with the largest flower were achieved at 100 mM PBZ. The flower scape had a big pith cavity at 100 mM PBZ followed by reducing its fresh weight, which may be considered as a negative point due to making flower scape more fragile. Overall, application of PBZ improved the cultivation of *N. tazetta*, and molecular analysis can support the role of PBZ to produce genetically modified plants with greater yield.

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