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Exogenous Gibberellic Acid and Paclobutrazol Enhance the Physiological Status and Photosynthetic Capacity of Two Cultivars of Potted Roses under Drought Stress

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ABSTRACT

Plant growth regulators (PGRs) can modify plant responses to drought stress. This study evaluated the effects of paclobutrazol (PBZ) and gibberellic acid (GA_3) on the growth, physiological, and biochemical responses of two miniature rose cultivars, "Sanaz-e-Sourati" and "Sanaz-e-Zard", under contrasting irrigation regimes. The experiment was conducted as a three-factor factorial experiment arranged in a randomized complete block design (RCBD) with five blocks. The factors consisted of six exogenous treatment regimes (GA_3, PBZ, simultaneous PBZ + GA_3, sequential PBZ and GA_3 at a 24-h interval, water spray, and an untreated control), two cultivars, and two irrigation regimes (80% and 20% of field capacity), giving 120 experimental units. Drought stress reduced several growth and physiological traits; in Sanaz-e-Sourati, flower bud number decreased from 5.9 to 3.2 buds per plant (approximately 45%), and photosynthetic rate decreased by approximately 28% under stress. Responses to PBZ- and GA_3-containing treatments depended on cultivar and irrigation regime, with treatment-specific differences in plant height, chlorophyll, proline, and catalase activity. These findings indicate that the effects of PBZ and GA_3 on drought responses in potted miniature roses are strongly context dependent and should be interpreted through the significant treatment × cultivar × irrigation interactions.

Keywords: Chlorophyll, Drought stress, Hormone treatments, Plant growth, *Rosa hybrida*.

INTRODUCTION

Climate change has contributed to a progressive increase in global temperatures (increased 1.8 – 4 °C by 2100), which in turn has exacerbated water scarcity in many regions (Ozturk *et al.,* 2021). Drought stress a major abiotic constraint significantly limits the growth and development of numerous plant species. This is especially pronounced in ornamental plants, including miniature

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roses (*Rosa* spp.), which are particularly susceptible to reduced water availability (Cai *et al.,* 2014). A total of thirty- five horticultural classes have been established for the description of rose cultivars that share broad characteristics with respect to growth habit, floral display, or genetic background. Miniature roses are compact rose cultivars whose plant height generally ranges from approximately 30 to 60 cm and that produce flowers in diverse colors (Anderson, 2006). Drought stress causes physiological reactions of plants to endure drought stress including leaf wilting, diminished leaf area, and leaf abscission (Ahmad, 2010). Drought stress not only impacts plant morphological processes but also modifies the physiological, phytochemical and molecular in rose (Zarif *et al.,* 2025). Major outcome of water deficiency is the accumulation of reactive oxygen species (Ozturk *et al.,* 2021) within plant tissues. These highly reactive molecules are broadly categorized into two groups: radical species, like superoxide ($O_2•^-$), alkoxy radicals (RO•), and hydroxyl radicals (•OH); and non-radical forms, which including singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2) (Anjum *et al.,* 2017; Ibrahim *et al.,* 2019).In reaction to drought stress, plants react to abiotic stresses through hormonal activities (Liu *et al.,* 2019). regulators PGRs serve a critical role in the regulation of plant growth and development. The function of phytohormone like Gibberellic acid (GA) is not well established under drought stress. Because, the crucial functions of GAs are proven and reported for plants across all stages of their life cycle, such as, cell elongation, flowering, seed germination and elimination of dormancy (Ashraf *et al.,* 2002; Rady *et al.,* 2019). Many studies have claimed reduced signaling and level of GA under stress (Ahmad, 2010; Yang *et al.,* 2013 and Abdulaziz *et al.,* 2020). Also, enough evidence exists regarding the effects of GA_3 treatments on various plant growth and physiological processes under drought stress (Kaya *et al.,* 2006, Al Mahmud *et al.,* 2019). GA balanced both oxidative responses and enzymatic antioxidant defenses, thereby mitigating the harmful consequences brought on by abiotic stress (Khan *et al.,* 2012). Contrary to expectations, few studies are reported the important roles of GAs in drought stress response and adaptation, especially in miniature rose. PBZ as a triazole-based compound, disrupts gibberellin production within plants by targeting kaurene oxidase, thereby preventing the conversion of kaurene into kaurenoic acid. PBZ has been found to improve plant tolerance to drought stress and reduce cold sensitivity in plants (Kamran *et al.,* 2018). PBZ also increases the relative water content of the leaf surface, reduces evaporation-transpiration, and causes resistance in plants against biotic and abiotic stresses (Desta and Amare, 2021). This study present new research to understand the effect of PBZ and GA_3 exogenous

treatments on potted roses under drought stress. The knowledge aimed through this study would provide a deep understanding into the role of GA_3 alone and with PBZ treatments, which may play a crucial role in growth improvement for drought stress tolerance.

Therefore, the objective of this study was to evaluate the individual and combined effects of exogenously applied GA_3 and PBZ on the growth, physiological, and biochemical responses of two miniature rose cultivars under normal and drought-stress conditions. We further aimed to determine whether exogenous treatment, cultivar, and irrigation regime interact in determining the drought responses of potted miniature roses.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

The current investigation was carried out in the research greenhouse of the Faculty of Agriculture at Tarbiat Modares University during the period from April to June 2021. The greenhouse was maintained at an average daily temperature of 22–24 °C and an average relative humidity of approximately 65%, and the plants were exposed to natural daylight. The experiment was conducted as a three-factor factorial experiment arranged in a randomized complete block design (RCBD) with five blocks. The factors consisted of six exogenous treatment regimes, two miniature rose cultivars, and two irrigation regimes. Each block contained all 24 treatment combinations, resulting in a total of 120 experimental units. Each experimental unit consisted of a 2-L pot filled with a growing medium composed of leaf compost, agricultural soil, and decomposed manure. The cultivars were “Sanaz-e-Sourati” (pink flowers) and “Sanaz-e-Zard” (yellow flowers). Irrigation treatments were non-stress irrigation at 80% of field capacity and severe water-deficit stress at 20% of field capacity. Six exogenous treatment regimes were evaluated: water spray, 0.018 g L^{-1} PBZ, 1.2 g L^{-1} GA_3, simultaneous application of 0.018 g L^{-1} PBZ + 1.2 g L^{-1} GA_3, sequential application of PBZ and GA_3 at a 24-h interval, and an untreated control. Although PBZ is commonly applied as a soil drench, foliar application has also been reported in herbaceous and woody plants, including wheat (Nagar *et al.,* 2021), sugar beet (Pengjie *et al.,* 2025), and olive (Ajmi *et al.,* 2020). In this experiment, PBZ was applied to the plant canopy.

Growth/yield and photosynthetic measurements were obtained from all five blocks, whereas total chlorophyll, proline, soluble solids, and catalase activity were measured in three of the five blocks.

Measurement of Growth Parameters and Soil Water Content

The current study assessed the growth characteristics of the plants, including plant height, number of flower buds, and both fresh and dry weights of leaves and branches. Weight measurements were conducted over three trials using a digital scale with an accuracy of 0.001 g. To determine the fresh weight of the aerial parts (shoots and leaves), they were carefully severed from the soil surface of the pot and their weight was recorded using a digital weighing device (UX420H; Shimadzu Corp., Kyoto, Japan). The dry weight was determined after drying the plant material in a temperature-regulated oven at 50 °C for 48 h to constant weight. Field capacity and irrigation volume were determined gravimetrically. Field capacity was determined 24 h after irrigation, and the TDR device was calibrated against this gravimetric determination. Irrigation volumes were subsequently determined gravimetrically according to the target irrigation regimes of 80% and 20% of field capacity (Noborio, 2001).

Measurements of photosynthesis

Photosynthetic measurements were performed on two occasions during the early flowering stage, on sunny days between 09:00 and 12:00, at an ambient measurement temperature of approximately 25 ± 2 °C. Each experimental pot contained one plant, and three upper fully expanded leaves from that plant were used for the measurements. Net photosynthetic rate and stomatal conductance were measured using a LI-COR 6400 photosynthesis system (LI-COR Inc., Lincoln, NE, USA), following the cited protocol.

Analysis of Chlorophyll (Chl) Content

Total chlorophyll content was determined in the most recently matured leaves according to Arnon (1949). Plant sampling was conducted three times. For pigment extraction, 50 mg of frozen, ground leaf tissue was homogenized in 1 mL of cold 95% methanol and vortexed at 25 ± 2 °C. Samples were centrifuged at 4,025 × g for 10 min, and the supernatant was transferred to a new microtube. The extraction was repeated twice and the supernatants were combined. Absorbance was measured at 665 and 652 nm using a microplate reader.

Analysis of Proline Content

For the determination of proline levels in leaves, 200µl of ethanolic extract was combined with 400 µl of the reaction mixture consisting of 1% (w/v) ninhydrin, 20% (v/v) ethanol, and 60% (v/v)

glacial acetic acid, which was then heated in a Bain-Marie at 95°C for 20 min. Following cooling to ambient temperature, the absorbance of the specimens was recorded at 520 nm employing a microplate reader. The proline content was determined based on a standard curve, as this methodology was described by researchers Carillo and Gibon, (2011). The present study utilized an ethanol-based extraction method to measure the soluble solid content (SSC) of leaf samples. Specifically, 40 mg of powdered frozen leaf was mixed with 0.5 mL of 80% (v/v) ethanol and heated at 80°C for 20 min in a Bain-Marie to obtain a hot ethanol extract. This extraction process was repeated four times and the resulting supernatants were collected in a second microtube. The SSC was determined in microplate format using the phenol sulfuric acid method proposed by Masuko *et al.,* (2005). About 150 microliters of concentrated sulfuric acid and 30 microliters of 5% phenol were admixed rapidly to a micro-tube containing 50 microliters of ethanol extract. The micro-tubes were then incubated for five minutes at 90°C in a Thermo block followed by cooling at room temperature for another five minutes. The optical density was measured at 490 nm by means of a microplate reader. The total amount of dissolved solids was determined by calculating against the standard curve.

Analysis of catalase enzyme activity

The current study assessed the catalase activity (CAT-EC 1.11.1.6) in leaves using the decomposition rate of hydrogen peroxide (H_2O_2) in the enzyme-substrate and the corresponding decrease in absorbance at 240 nm, according to the method Li and Schellhorn, (2007). Specifically, 30 µl of the enzyme extract was admixed with 150 µl of 50 mM potassium phosphate buffer (pH=7) and the resulting mixture was incubated for one minute at room temperature condition. This approach allowed for an accurate assessment of catalase activity and would be useful for future studies in this field.

Statistical Analysis

SAS software was used for statistical analysis. The experiment was analyzed as a three-factor factorial arrangement in a randomized complete block design, with block and the fixed effects of exogenous treatment (A; six levels), cultivar (B; two levels), irrigation regime (C; two levels), and their two- and three-way interactions included in the model. Total chlorophyll, proline, soluble solids, and catalase activity were analyzed using the three sampled blocks. Photosynthetic rate was measured on two occasions and was square-root transformed [$\sqrt{(x + 0.5)}$] before inferential

analysis. For the LI-COR data, time was included in the model; the between-subject effects of A, B, C, and their interactions were tested using the Type III mean square for block × A × B × C as the error term. Mean comparisons for cultivar and irrigation were based on this error term. Untransformed photosynthetic means averaged across the two measurement occasions are presented in the text and figures for biological interpretation, whereas significance tests and mean-separation letters are based on the transformed analysis. Residuals were assessed for normality before analysis of variance. Mean comparisons for the remaining traits were performed at $P \leq 0.05$ using the least significant difference (LSD) procedure. When a higher-order interaction was significant, it was decomposed using slice comparisons (simple-effect analysis), in which treatment-combination means are compared within fixed levels of another factor rather than interpreting the corresponding marginal main effects (Winer, 1971; Maxwell et al., 2018). For significant A × B × C interactions, exogenous treatment × irrigation treatment-combination means were therefore compared separately within each cultivar. Graphs were prepared in Excel.

RESULTS

Plant height

The exogenous treatment × cultivar × irrigation interaction (A × B × C) was significant for plant height ($P < 0.01$; Table 1). To decompose this significant three-way interaction, exogenous treatment × irrigation combination means were compared separately within each cultivar. In Sanaz-e-Sourati, GA3 under non-stress irrigation produced the greatest mean height (34.8 cm). Under drought stress, simultaneous PBZ + GA3 produced a mean height of 29.2 cm and was among the highest treatment combinations. In Sanaz-e-Zard, simultaneous PBZ + GA3 under non-stress irrigation produced the greatest height (43.6 cm), whereas under drought stress the sequential PBZ–GA3 treatment had the numerically greatest mean (27.0 cm). These patterns confirm that treatment effects on height depended jointly on cultivar and irrigation regime (Fig. 1).

Table 1. Table 1. Analysis of variance for the effects of exogenous treatment, cultivar, irrigation regime, and their interactions on growth, physiological, and biochemical traits of the miniature rose cultivars Sanaz-e-Sourati and Sanaz-e-Zard under non-stress (80% field capacity) and drought-stress (20% field capacity) irrigation.

Source of variation	Degree of freedom	Mean squares								
		Plant height	Number of flower buds per plant	Total fresh weight	Dry weight	Total chlorophyll	Proline	Catalase	Soluble solide	Photosynthetic rate
Block	4†	46.90ns	3.35ns	48.92*	5.80ns	2.32ns	0.22*	0.20ns	148.96ns	0.82**
Exogenous treatment (A)	5	286.47**	6.13*	17.98ns	2.28ns	18.87**	0.35**	0.28*	2624.53*	0.17ns
Cultivar (B)	1	116.03ns	61.63**	4.25ns	3.15ns	0.20ns	3.63**	0.002ns	23747.78**	8.69**
Irrigation (C)	1	853.33**	93.63**	410.29**	101.92**	2.16ns	1.39**	1.72**	601.64ns	3.30**
A×B	5	23.17ns	3.39ns	20.46ns	5.95ns	2.95ns	0.30**	0.40**	3813.70**	0.17ns
A × C	5	101.35*	4.43ns	14.88ns	5.62ns	11.26*	0.74**	0.70**	3017.44*	0.08ns
B × C	1	28.03ns	48.13**	17.47ns	3.21ns	51.27**	1.38**	0.0007ns	17.13ns	0.08ns
A × B × C	5	168.05**	1.25ns	13.81ns	7.96ns	11.48*	0.49**	0.65**	1424.08ns	0.04ns
CV (%)	-	22.23%	39.79%	29.62%	28.63%	20.84%	26.42%	35.64%	39.53%	29.67%

*, ** and ns indicate P ≤0.05, P ≤ 0.01 and non-significance, respectively. CV = coefficient of variation. A = exogenous treatment (GA_3, PBZ, simultaneous PBZ + GA_3, sequential PBZ and GA_3, water spray, and untreated control); B = cultivar (Sanaz-e-Sourati and Sanaz-e-Zard); C = irrigation regime (non-stress, 80% field capacity; drought stress, 20% field capacity). †Block df = 2 for total chlorophyll, proline, catalase activity, and soluble solids because these traits were measured in three of the five blocks; block df = 4 for traits measured in all five blocks. Photosynthetic-rate ANOVA was conducted on $\sqrt{(x + 0.5)}$-transformed data. The between-subject effects were tested using the Type III MS for block × A × B × C as the error term. Untransformed photosynthetic means averaged across the two measurement occasions are presented in the text and figures.

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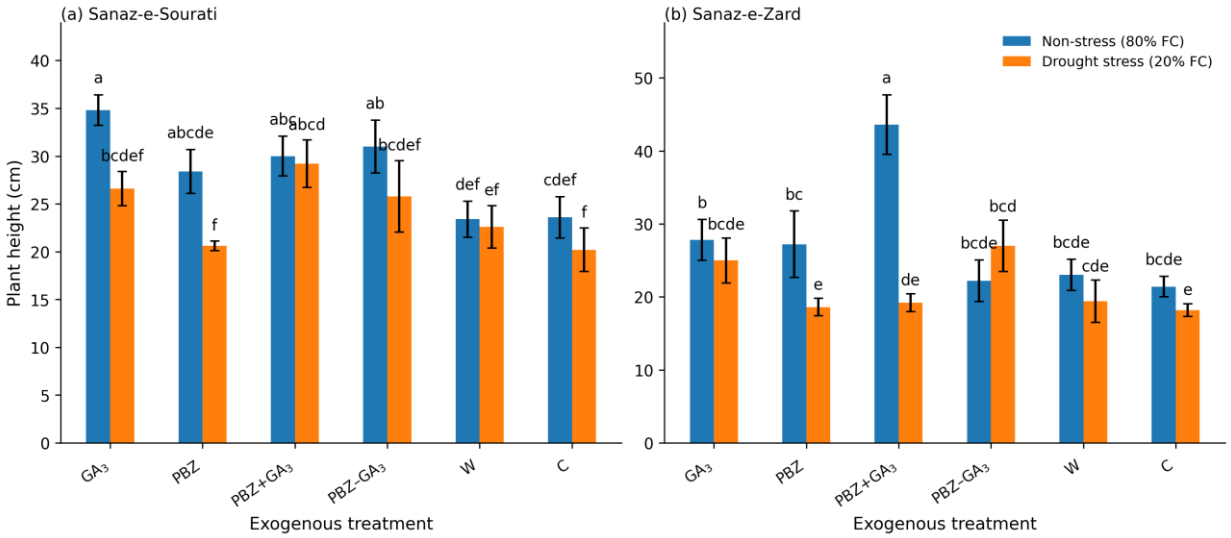

Figure 1. Decomposition of the significant exogenous treatment × cultivar × irrigation interaction for plant height. Exogenous treatment × irrigation combination means are presented separately within Sanaz-e-Sourati (a) and Sanaz-e-Zard (b). Bars show means ± SE from five blocks. Within each cultivar slice, means sharing at least one letter are not significantly different by LSD at $P \leq 0.05$. Non-stress irrigation = 80% field capacity; drought stress = 20% field capacity. Treatment abbreviations: GA_3 = gibberellic acid; PBZ = paclobutrazol; PBZ + GA_3 = simultaneous application; PBZ–GA_3 = sequential application at a 24-h interval; W = water spray; C = untreated control.

Number of flower buds per plant

The number of flower buds per plant showed a significant exogenous-treatment main effect ($P \leq 0.05$) and a significant cultivar × irrigation interaction (B × C; $P \leq 0.01$), whereas the exogenous treatment × cultivar (A × B), exogenous treatment × irrigation (A × C), and three-way (A × B × C) interactions were not significant (Table 1). Because none of the interactions involving exogenous treatment was significant, its significant main effect was interpreted across cultivars and irrigation regimes; cultivar and irrigation were interpreted through their significant B × C interaction. Across cultivars and irrigation regimes, PBZ produced the greatest overall mean number of flower buds (4.7 buds per plant). For the B × C interaction, Sanaz-e-Sourati declined from 5.9 buds per plant under non-stress irrigation to 2.87 under drought stress, whereas Sanaz-e-Zard showed similar means under non-stress (3.2) and drought-stress (2.7) irrigation. Thus, drought reduced flower-bud number significantly in Sanaz-e-Sourati but not in Sanaz-e-Zard (Fig. 2).

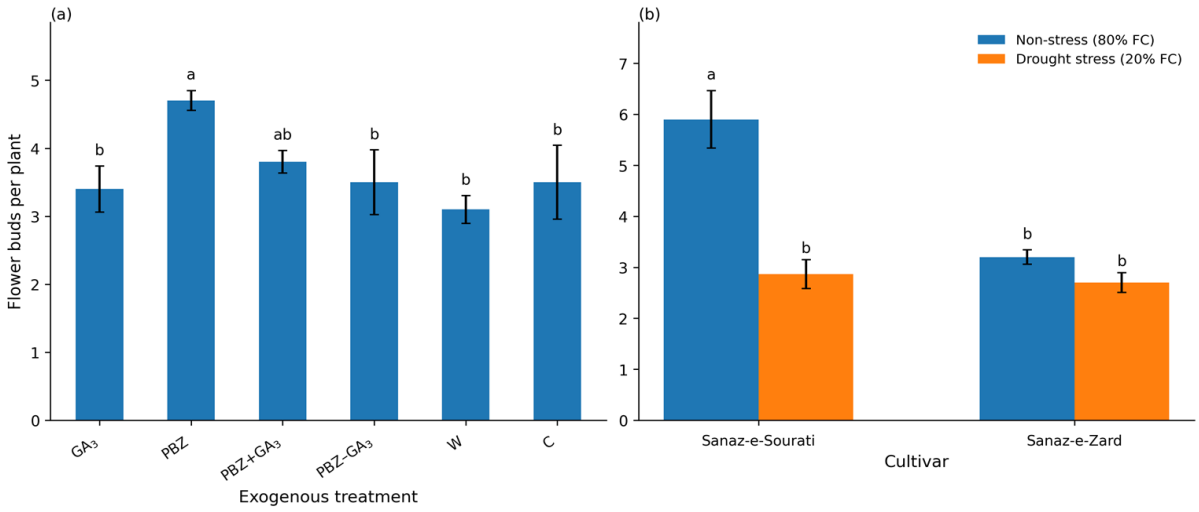

Figure 2. Number of flower buds per plant. (a) Significant exogenous-treatment main effect, presented because A × B, A × C, and A × B × C were not significant; (b) significant cultivar × irrigation interaction. Bars show means ± SE from five blocks. Means sharing at least one letter are not significantly different by LSD at $P \leq 0.05$. The two cultivars are Sanaz-e-Sourati and Sanaz-e-Zard; non-stress irrigation = 80% field capacity and drought stress = 20% field capacity. Treatment abbreviations: GA_3 = gibberellic acid; PBZ = paclobutrazol; PBZ + GA_3 = simultaneous application; PBZ–GA_3 = sequential application at a 24-h interval; W = water spray; C = untreated control.

Biomass

Drought stress significantly reduced both total fresh and total dry biomass (Table 1). No interaction among exogenous treatment, cultivar, and irrigation regime was significant for either biomass trait; therefore, the significant irrigation main effect was interpreted. Total fresh weight decreased from 15.68 g under non-stress irrigation to 11.98 g under drought stress (23.6% reduction), while total dry weight decreased from 7.87 to 6.03 g (23.4% reduction; Fig. 3).

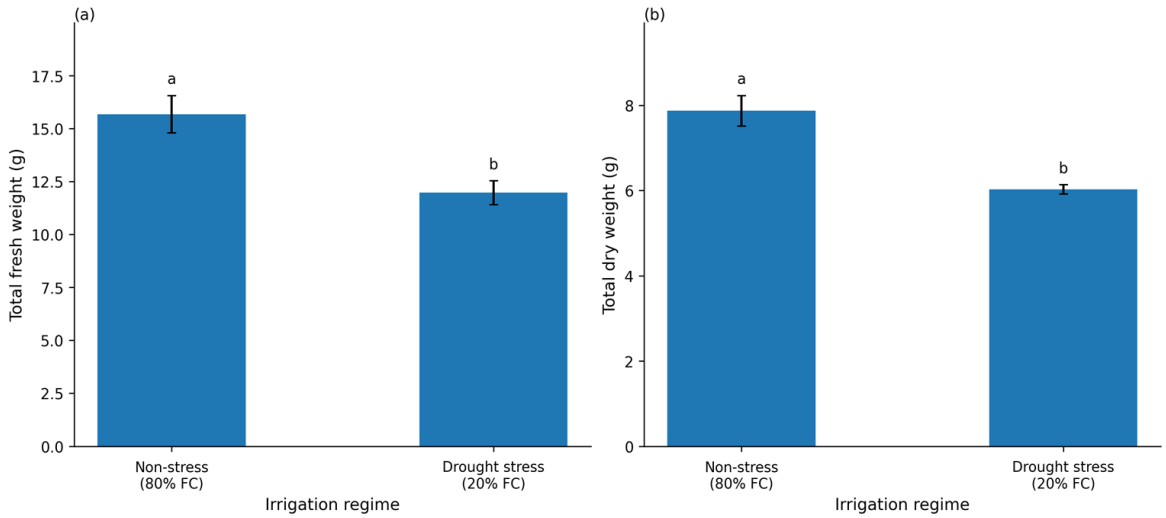

Figure 3. Significant irrigation main effect on total fresh weight (a) and total dry weight (b) of miniature rose plants; no interaction among exogenous treatment, cultivar, and irrigation regime was significant for either trait. Bars show means ± SE from five blocks. Different letters indicate significant differences by LSD at $P \leq 0.05$. Non-stress irrigation = 80% field capacity; drought stress = 20% field capacity.

Total chlorophyll content

The exogenous treatment × cultivar × irrigation interaction was significant for total chlorophyll ($P < 0.05$; Table 1). Accordingly, the significant three-way interaction was decomposed by comparing exogenous treatment × irrigation treatment-combination means separately within each cultivar. In Sanaz-e-Sourati, the greatest mean was observed for water spray under non-stress irrigation (12.79 mg g^{-1} FW), whereas under drought stress PBZ had the greatest numerical mean (9.83 mg g^{-1} FW). In Sanaz-e-Zard, the largest chlorophyll means occurred under drought stress with water spray (13.19 mg g^{-1} FW) and PBZ (12.46 mg g^{-1} FW), both belonging to the highest significance group (Fig. 4).

Proline

The exogenous treatment × cultivar × irrigation interaction was significant for proline content ($P < 0.01$; Table 1). Accordingly, the significant three-way interaction was decomposed by comparing exogenous treatment × irrigation combination means separately within each cultivar. In Sanaz-e-Sourati, simultaneous PBZ + GA3 under drought stress produced the greatest numerical proline mean (1.03 mg g−1 FW). In Sanaz-e-Zard, the highest proline values under drought stress were obtained with the sequential PBZ–GA3 treatment (2.43 mg g−1 FW) and simultaneous PBZ + GA3 (2.29 mg g−1 FW), which belonged to the highest significance group (Fig. 5).

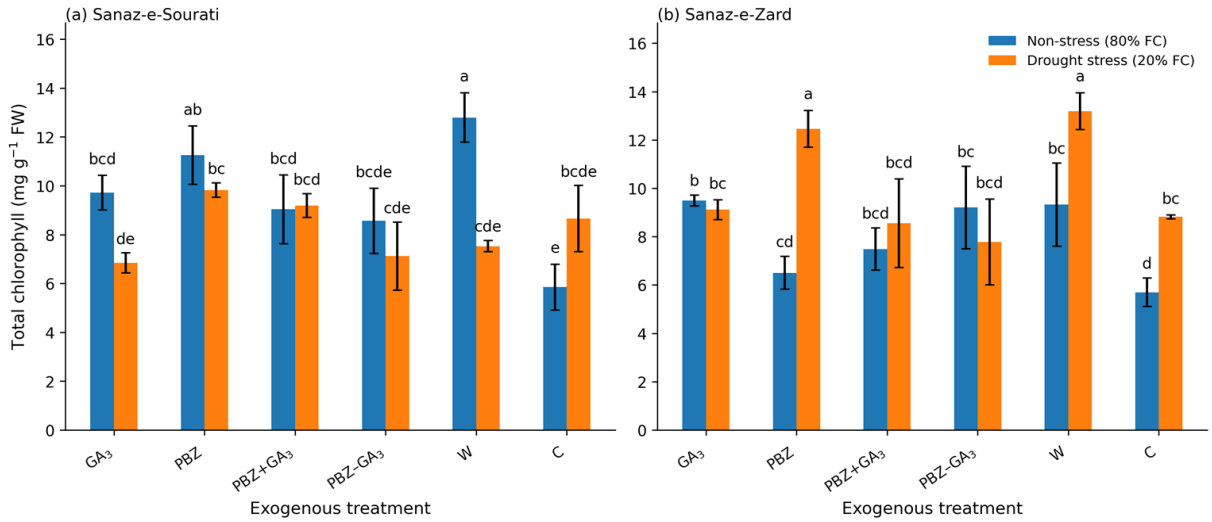

Figure 4. Decomposition of the significant exogenous treatment × cultivar × irrigation interaction for total chlorophyll content. Exogenous treatment × irrigation combination means are presented separately within Sanaz-e-Sourati (a) and Sanaz-e-Zard (b). Bars show means ± SE from three sampled blocks. Within each cultivar slice, means sharing at least one letter are not significantly different by LSD at $P \leq 0.05$. Non-stress irrigation = 80% field capacity; drought stress = 20% field capacity. Treatment abbreviations: GA_3 = gibberellic acid; PBZ = paclobutrazol; PBZ + GA_3 = simultaneous application; PBZ–GA_3 = sequential application at a 24-h interval; W = water spray; C = untreated control.

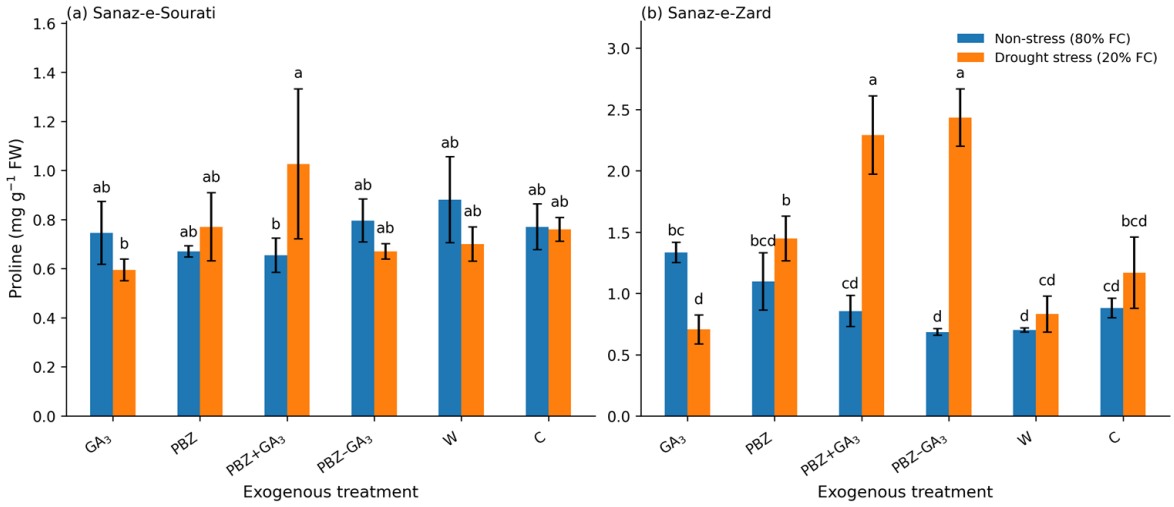

Figure 5. Decomposition of the significant exogenous treatment × cultivar × irrigation interaction for proline content. Exogenous treatment × irrigation-combination means are presented separately within Sanaz-e-Sourati (a) and Sanaz-e-Zard (b). Bars show means ± SE from three sampled blocks. Within each cultivar slice, means sharing at least one letter are not significantly different by LSD at $P \leq 0.05$. Non-stress irrigation = 80% field capacity; drought stress = 20% field capacity. Treatment abbreviations: GA_3 = gibberellic acid; PBZ = paclobutrazol; PBZ + GA_3 = simultaneous application; PBZ–GA_3 = sequential application at a 24-h interval; W = water spray; C = untreated control.

Soluble solid content (SSC)

Soluble solid content (SSC) was affected by significant exogenous treatment × cultivar (A × B; $P \leq 0.01$) and exogenous treatment × irrigation (A × C; $P \leq 0.05$) interactions, whereas the three-way interaction was not significant (Table 1). Because A × B × C was not significant, the two significant two-way interactions were interpreted separately. For A × B, simultaneous PBZ + GA3 produced the highest SSC in Sanaz-e-Sourati (127.48 mg/wet weight) but the lowest SSC in Sanaz-e-Zard (34.65 mg/wet weight). For A × C, PBZ under non-stress irrigation gave the highest mean (113.59 mg/wet weight), while the sequential PBZ–GA3 treatment increased from 38.73 under non-stress irrigation to 90.48 mg/wet weight under drought stress (Fig. 6).

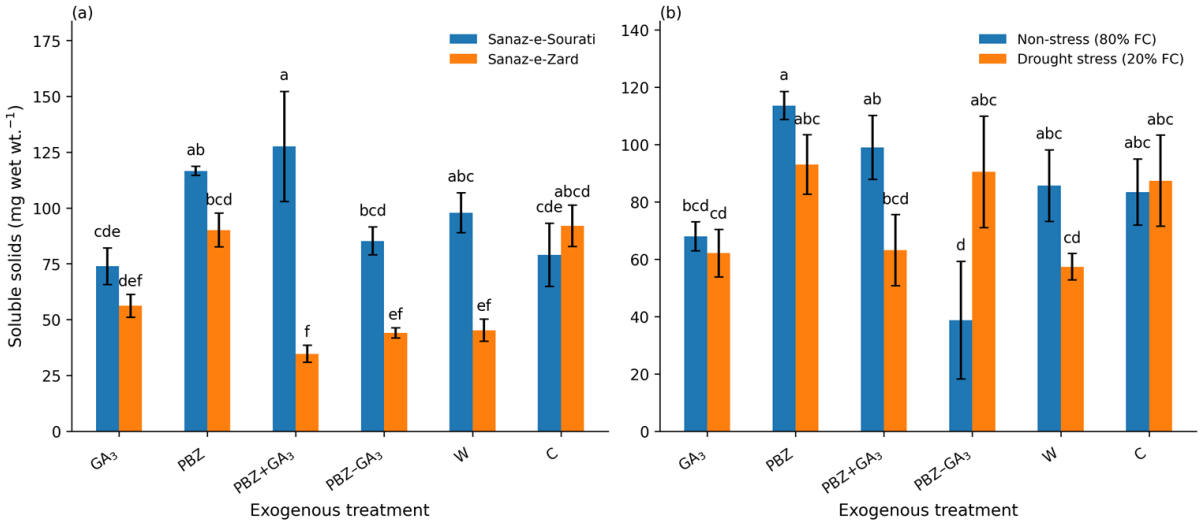

Figure 6. Significant two-way interactions for soluble solid content (SSC). Because A × B × C was not significant, (a) the exogenous treatment × cultivar interaction is presented averaged across irrigation regimes and (b) the exogenous treatment × irrigation interaction is presented averaged across cultivars. Bars show means ± SE from three sampled blocks. Means sharing at least one letter are not significantly different by LSD at $P \leq 0.05$. Cultivars are Sanaz-e-Sourati and Sanaz-e-Zard; non-stress irrigation = 80% field capacity and drought stress = 20% field capacity. Treatment abbreviations: GA_3 = gibberellic acid; PBZ = paclobutrazol; PBZ + GA_3 = simultaneous application; PBZ–GA_3 = sequential application at a 24-h interval; W = water spray; C = untreated control.

Catalase enzyme

The exogenous treatment × cultivar × irrigation interaction was significant for catalase activity ($P < 0.01$; Table 1). Accordingly, the significant three-way interaction was decomposed by comparing exogenous treatment × irrigation combination means separately within each cultivar. In Sanaz-e-Sourati, GA3 under non-stress irrigation produced the highest catalase activity (1.66 µmol mg−1 min−1), whereas under drought stress PBZ (1.21) and simultaneous PBZ + GA3 (1.13 µmol mg−1 min−1) were among the highest

treatments. In Sanaz-e-Zard, the sequential PBZ–GA3 treatment under drought stress produced the highest mean catalase activity (1.50 µmol mg−1 min−1; Fig. 7).

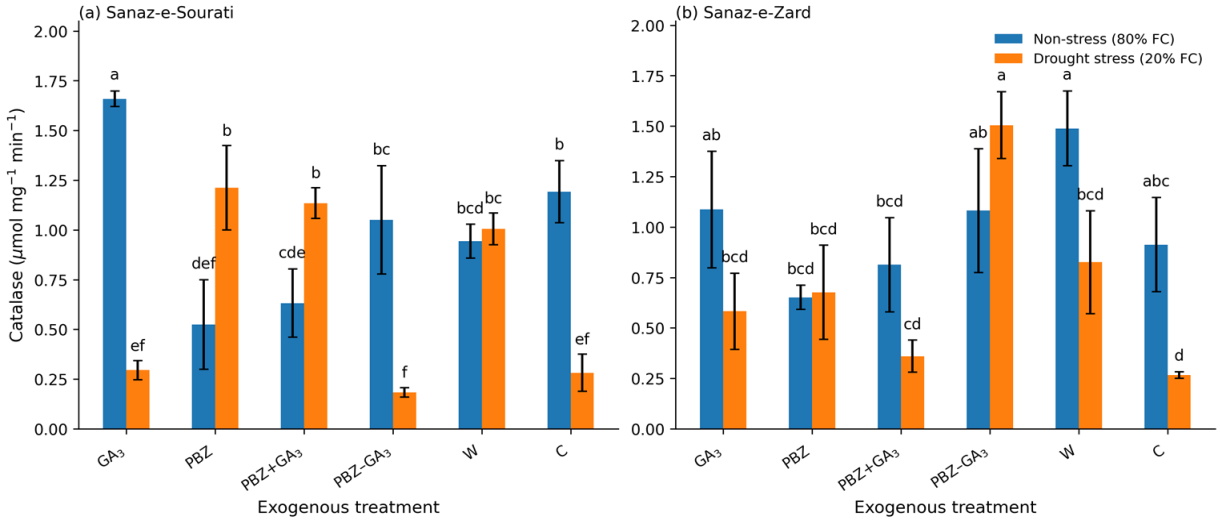

Figure 7. Decomposition of the significant exogenous treatment × cultivar × irrigation interaction for catalase activity. Exogenous treatment × irrigation combination means are presented separately within Sanaz-e-Sourati (a) and Sanaz-e-Zard (b). Bars show means ± SE from three sampled blocks. Within each cultivar slice, means sharing at least one letter are not significantly different by LSD at $P \leq 0.05$. Non-stress irrigation = 80% field capacity; drought stress = 20% field capacity. Treatment abbreviations: GA_3 = gibberellic acid; PBZ = paclobutrazol; PBZ + GA_3 = simultaneous application; PBZ–GA_3 = sequential application at a 24-h interval; W = water spray; C = untreated control.

Photosynthetic parameters

Photosynthetic rate was significantly affected by cultivar ($P \leq 0.01$) and irrigation regime ($P < 0.01$), whereas exogenous treatment and the A × B, A × C, B × C, and A × B × C interactions were not significant in the transformed analysis (Table 1). Because all interactions among exogenous treatment, cultivar, and irrigation regime were nonsignificant, the significant cultivar and irrigation main effects were interpreted as marginal effects. Untransformed marginal means averaged across the two measurement occasions were 1.66 and 3.08 µmol CO2 m^{-2} s^{-1} for Sanaz-e-Sourati and Sanaz-e-Zard, respectively. Mean photosynthetic rate decreased from 2.75 under non-stress irrigation to 1.99 µmol CO2 m^{-2} s^{-1} under drought stress, corresponding to a 27.8% reduction (Fig. 8).

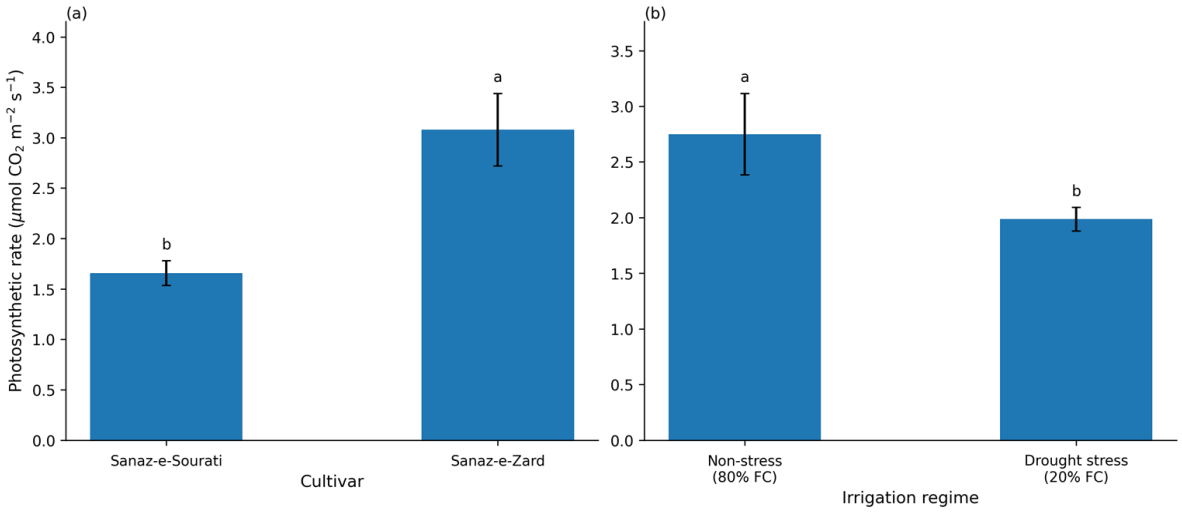

Figure 8. Significant main effects of cultivar (a) and irrigation regime (b) on photosynthetic rate, shown because all interactions among exogenous treatment, cultivar, and irrigation regime were nonsignificant. Means are averaged across the two measurement occasions. Bars show untransformed means ± SE; different letters indicate significant differences based on the √(x + 0.5)-transformed analysis using the Type III MS for block × A × B × C as the error term. The cultivars are Sanaz-e-Sourati and Sanaz-e-Zard; non-stress irrigation = 80% field capacity and drought stress = 20% field capacity.

DISCUSSION

Abiotic stress declines morphological, physiological and phytohormone concentrations in the plant (Abdulaziz et al., 2020). Drought stress, as abiotic stressor in horticulture and floriculture, criticaly reduce crop yeild and limit the growth. Plants have diffrrent stategieis to metigate the adverse effecs of the drought strees. PGRs, are effective in promoting generative and reproductive growth under water limmitation. Results showed Maximum height under normal irrigation was obtained with gibberellic acid, and under stress with combined PBZ + GA in Sanaz-e- Sourati (Fig. 1a). Several studies have shown that gibberellin increases plant high by inducing cell elongation. these findings are in GA+PBZ (Fig. 1b). PBZ and stress inhibit the synthesis of endogenous gibberellins. In stress conditions, the exogenous application of GA does not obviate the plant's need to sustain endogenous GA production from primary carbohydrate sources. (Yadav et al., 2021). Exogenous GA+PBZ treatment can regulate the endogenous levels of bioactive gibberellins in plants (Tonkinson et al., 1997). For this reason, the application of exogenous GA alongside PBZ modulated the impact of PBZ on plant morphology, specially plant hight under stress conditions. The results of variance analysis and mean comparisons indicated that the maximum number of

flower buds was observed in response to PBZ application (Tab. 1 and Fig. 2a). Paclobutrazol inhibits gibberellin biosynthesis and modifies the balance of other phytohormones, including cytokinins, changes that may contribute to its effects on plant architecture and stress responses (Desta & Amare, 2021; Maheshwari et al., 2022). Cytokinins are positive regulators of axillary-bud outgrowth and shoot branching (Kotov et al., 2021), which may increase the number of potential sites for reproductive development. The results indicate that PBZ treatment can aid in sustaining bud development under optimal conditions, while stress conditions can severely limit reproductive potential. This understanding highlights the importance of selecting appropriate management strategye like PBZ exogenouse treatment to optimize bud flower formation under drought strees condition. Also, our results revealed that the treatment of PBZ increased the chlorophyll content in Sanaz-e-Sourati and Sanaz-e-Zard cultivars under stress (Fig. 4). Abraham et al. (2008) conducted a study on the impact of PBZ application on the chlorophyll content of sesame plants. The authors concluded that the higher chlorophyll level in triazole-treated plants may be attributed to the influence of triazole on cytokinin levels. Previous research has shown that triazoles promote cytokinin biosynthesis, which in turn enhances chloroplast development, chloroplast number, and chlorophyll synthesis while preventing chlorophyll degradation (Khalil & Rahman, 1995). More recent evidence also indicates that PBZ can enhance photosynthetic pigment retention and antioxidant capacity under water-deficit conditions, although the magnitude of these responses varies among plant species (Maheshwari et al., 2022). Maintaining chlorophyll levels is crucial for sustaining photosynthesis, and the positive influence of PBZ indicates its potential in managing chlorophyll degradation during stressful periods. The results demonstrated a significant decrease in the photosynthesis rate, which dropped by 28% with increasing stress levels (Fig. 8). The photosynthesis rate is dependent on plant genetic background (genus, species and cultivars), leaf age and environmental conditions including light quality and quantity, temperature, available water and CO2 concentration (de Hoog, 2001). Comparing to cut roses, the speed of growth and development in miniature roses is relatively slow down which could be attributed to its low photosynthesis rate. Proline commonly accumulates in plants under drought and other osmotic stresses and contributes to osmotic adjustment, cellular homeostasis, redox balance, and protection against oxidative damage (Ghosh et al., 2022). Our results showed that the Sanaz-e-Sourati cultivar, gibberellic acid alone maximized proline under normal conditions, whereas the sequential application of PBZ and GA (one-day interval) (PBZ-GA) was superior

under stress (Fig. 5). I has been suggested that the promote in proline level in plants during stress may be attributed to change in the oxidation rate of proline to glutamate or its lack of involvement in protein synthesis as reported by Khan et al., (2007). Additionally, increasing SSC content is another strategy for managing water deficit in plants to enhance water-stress tolerance. The rise in SSC in reaction to water shortage stress can be attributed to less transport of these compounds from leaves under stress conditions, as well as slower consumption due to reduced growth and other changes like starch hydrolysis (Kameli & Lösel, 1996). Under both normal and water-stress conditions, our results showed that PBZ treatment enhanced SSC content in plants (Fig. 6). Previous studies have suggested that paclobutrazol treatment tends to increase the concentration of SSC (Gastol & Poniedzialek, 2004). This is agree with the results of a study by Setia et al., (1995), which reported that leaves of plants treated with paclobutrazol revealed higher chlorophyll and SSC content and remained longer than the control on intact plants. Plants under stress generally show concurrent ROS production and upregulated antioxidant defenses (Ozturk et al., 2021). It has been showed that an increase in catalase activity with increasing drought stress in alfalfa seedlings (Wang et al., 2009).Our results indicated the increase in catalase activity following PBZ and GA-PBZ exogenous treatments is agree with the findings of Nagar et al., (2021). Gibberellic acid modulated the oxidative stress processes and antioxidant enzyme activity, consequently suppressing the negative effect of abiotic stress (Khan et al., 2012). Studies showed that in addition to the antioxidant defense system, exogenous GA upregulated the glyoxalase system, which assisted the survival of wheat seedlings under drought stress (Moumita Al Mahmud et al., 2019). The findings suggest that implementing strategies to boost antioxidant enzyme activity, such as through PBZ-GA application, can improve plant resilience to drought stress. A recent study on Rosa hybrida similarly demonstrated that drought stress adversely affected chlorophyll content, biomass, photosynthetic performance, and antioxidant metabolism (Zhao et al., 2024). Consistent with these responses, recent work in Rosa centifolia showed that water deficit induced marked changes in proline accumulation and antioxidant-enzyme activities, including catalase, supporting an important role for osmotic adjustment and antioxidant defense in rose drought responses (Ahsan et al., 2025).

CONCLUSIONS

This study showed that the responses of miniature roses to PBZ and GA3 depended on cultivar and irrigation regime. Drought stress reduced biomass and photosynthetic rate and, in Sanaz-e-Sourati, markedly reduced the number of flower buds per plant. PBZ-containing treatments were associated with improved chlorophyll, proline, and catalase responses in specific cultivar–irrigation combinations. These results indicate that exogenous PBZ and GA3 can modify drought responses in potted miniature roses, but their effects should be interpreted in relation to cultivar and irrigation conditions rather than as uniform treatment main effects.

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