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Paclobutrazol Influences Vegetative and Reproductive Growth, Physiology and Quality of Watermelon Hybrids

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ABSTRACT

Paclobutrazol (PBZ) is a member of the triazole plant growth inhibitor group that is responsible for controlling plant height and inducing flowering. An experiment was, therefore, conducted to examine the foliar application of PBZ at various concentrations to control the vigorous growth of watermelon seedlings in a greenhouse. The first factor was the concentration of PBZ at 0, 250, 500, 750 and 1000 ppm as a foliar spray, and the second factor was hybrids of watermelon i.e. GE344, GE341 and SSH348. Foliar application of PBZ reduced plant height and unwanted vegetative growth in tested watermelon hybrids. The reduction in plant height for 250 ppm was almost 14.26% compared to the non-treated plants; however, 1,000 ppm, showed extremely the shortest plant. With the increase in PBZ concentration from 0 to 1,000 ppm, plant height and leaf area were reduced. Results also indicated that in all three hybrids the highest relative chlorophyll content, number of the female flowers, fresh and dry root weights, root diameter and TSS (% Brix) were found in the application of 250 ppm compared to other PBZ concentrations. Maximum fruit firmness was obtained at 500 ppm of PBZ. Among the hybrids, the GE344 produced significantly highest relative chlorophyll content, TSS%, fruit weight and fruit firmness. Considering the above results, 250 ppm of PBZ is recommended for commercial production of watermelon in greenhouse.

Key words: Paclobutrazol, concentration, watermelon, growth, quality

INTRODUCTION

Watermelon is an economically important fruit, widely cultivated and consumed around the world which belongs to Cucurbitaceae family (Liu *et al.*, 2018). Its global production in 2020 was 101,620,420 million tonnes (FAOSTAT, 2022). It contains vitamins, minerals, fiber, protein, carbohydrates, lycopene and phytonutrient (USDA, 2020). Under natural condition, the vegetative growth of watermelon is vigorous with abundant branch, resulting in a requirement of continual pruning to control the vegetative growth and reproductive development. Because of their long prostrate vines, they require a lot of garden space to provide a high harvest (Oga and Umekwe, 2016). Inside the greenhouse, seedling height and growth is a major challenging issue for most farmers. Vigorous growth of watermelon reduces the fruit size as well as increases labour cost and also time-consuming. Plant over growth can be controlled either by physical methods viz., pruning, branching, light, temperature, etc. or chemical ways such as growth regulators, particularly gibberellin inhibitors (flurprimidol, Paclobutrazol). Paclobutrazol (PBZ) is a gibberellin biosynthesis inhibitor, used in growth retardation on a wide hybrid of crops (Rademacher, 2015). Previously, its effects were reported in decreasing the plant growth (Abed, 2018; Flores *et al.*, 2018). In watermelon seedlings, foliar application of PBZ at 150 ppm was reported to decrease the plant height and leaf area by 23.4 and 16.2%, respectively. Hhowever, the chlorophyll content increased by 26.5%, compared to the control (Flores *et al.*, 2018). Moreover, Abed (2018) also disclosed that foliar application of PBZ at higher

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concentration (400 and 600 mg/l) reduced terminal growth of lettuce. Early studies showed that plants treated with PBZ had bigger roots, promoted root characteristics and increased the root growth (Kamran *et al.*, 2018). Increased root growth by PBZ is associated with increased level of endogenous cytokinin (Desta and Amare, 2021). The beneficial role of PBZ as a growth retardant has been studied by numerous researchers in fruit crops. To the best of knowledge, no published literature is available dealing with the impacts of PBZ in controlling plant and fruit growth, enhancing fruit quality in watermelon under greenhouse condition. Given this fact, it was critically important to assess the impact of PBZ in controlling plant growth, enhancing fruit growth and increasing fruit quality. So, the current study was conducted to prove the hypothesis that the PBZ could control the vigorous growth of watermelon plants by altering the physiological changes, induce flowering, fruit setting and improve fruit quality.

MATERIALS AND METHODS

This experiment was conducted at greenhouse of Farm 15, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malaysia which is located at latitude of 2°59'01.8"N and a longitude of 101°43'57.1"E at an elevation of about 45 m from sea level. It was carried out during the period of February to April, 2020.

Watermelon seeds of F_1 hybrids (GE341 Red Delight, GE344 Mellow Oblong and SSH348 Oval Yellow) were collected from Sin Seng Huat Seeds Sdn Bhd Company (Reg No: 274553 D) in Malaysia and sown in seedling tray filled with peat moss prior to hydro priming for overnight. At 14 days after sowing, the seedlings were transferred to poly bags (40.64 × 40.64 cm) filled with coco peat, maintaining distance 50×80 cm between one another. The plants were irrigated five times a day with automated fertigation system.

The treatments were organized in a randomized complete block design with factorial combinations with three replications. The treatments consisted of three F_1 watermelon hybrids (GE344 Mellow Oblong, GE341 Red Delight and SSH348 Oval Yellow) and five foliar concentrations (0, 250, 500, 750 and 1000 ppm) of PBZ [(PP333-TC) (2RS, 3RS)-1-(4-Chlorophenyl)-4,4-dimethy1-2-(1H-1,2,4-triazol-1-yl) pentan-3-ol] containing 95% active ingredient. The PBZ solutions were prepared by adding the appropriate amount of powder in 95% ethanol and mixed with a magnetic stirrer. Plants were sprayed with different concentrations of PBZ using a handheld sprayer until runoff occurred (two weeks after transplanting), while control plants were treated with distilled water of equal volume.

Plant height and internode length data were measured at harvest with measuring tape in cm, while the leaf area/plant was measured using a leaf area meter (Model Li-3100, LiCOR, Inc., Lincoln, Nebraska, USA) and expressed as cm^2. The greenness of leaf was measured non-destructively using a SPAD meter (SPAD-502 plus, Konika Minolta Optic, Inc, Japan) from the fully extended young leaves at 15 days after treatments. Data on number of female flowers/plant (average of three plants/treatment) were also counted from first female flower appearance. After harvest, shoot dry matter and root dry matter were measured by oven-drying of fresh shoot and root sample at 70° until constant weight was obtained. Fruit weight (kg), fruit firmness (Newton) and total soluble solids (% Brix) content were measured in the laboratory after harvest. Fruit weight was measured immediately after harvest and weighed by electronic weighing scale and was expressed in kilogram (kg). Total soluble solids (TSS%) content in the fruits was assessed at 25°C with digital Refractometer (Pal-1, Atago Co., Tokyo, Japan) and firmness was measured by using a computer connected Instron, Universal Testing Machine (Model 5543 P5995 USA). Separated roots from the soil were washed to measure root diameter (mm), and root length density (cm) by using root scanner (Epson Flatbed Scanner 1680, WinRHIZO software).

Collected data were subjected to a two-way analysis of variance (ANOVA) using the statistical analysis software (SAS 9.4). Difference between the treatment means was compared using least significant deference (LSD) at 5% level. The data for all response variables were presented as means of three replications ± standard errors.

RESULTS AND DISCUSSION

Plant height decreased with the increase in

PBZ concentration (Table 1). The tallest (417.35 cm) plant was observed in control treatment as against the shortest (240.93 cm) at 1000 ppm of PBZ treatment. Plant height decreased by 14.38, 33.33, 38.60 and 42.44% at 250, 500, 750 and 1000 ppm of PBZ, respectively, against control. The hybrid SSH348 performed the best 311.80 cm in terms of plant height which was statistically at par with GE344 (311.64 cm) hybrid, but 1.30% higher than the GE341 hybrid. Similar results reported that PBZ decreased plant height (Soumya *et al.*, 2017), and leaf area (Desta and Amare, 2021) in cucurbits, while increased chlorophyll content (Xia *et al.*, 2018). The present study confirmed these claims as PBZ had a huge effect on plant height, number of branches, root and shoot growth of *Citrullus lanatus*, with the exogenous application of PBZ. The PBZ-treated plants effectively retarded vegetative growth, which was probably due to the inhibition of cell elongation in the stem. The impact of the PBZ on plant growth had increased with increasing concentration. Thus, it would appear that using PBZ would reduce pruning practices, especially in greenhouse. PBZ was demonstrated to reduce plant height in a range of crops, including cucumber, squash, melon and watermelon (Flores *et al.*, 2018) and tomato (Pal *et al.*, 2016). The shorter stems noted in the PBZ treated plants were most probably due to the systematic inhibitory activity of gibberellins (GA) biosynthesis, which were plant growth regulators effective in shoot elongation (Binenbaum *et al.*, 2018).

A progressive decrease in internode length was observed with increasing concentration of PBZ, where 250, 500, 750 and 1000 ppm resulted in 29, 43, 55 and 54% shorter internode than the control treatment. Maximum internode length was observed at GE344 hybrid (7.30 cm) as compared to the minimum in GE341 (6.79 cm), which was statistically similar with SSH348 (7.01 cm). The results showed that all concentrations of PBZ reduced the length of internode of all three hybrids. The highest length of internode was recorded in non-treated control. This result is in agreement with those reported in maize (Kamran *et al.*, 2018), and rice (Dorairaj and Ismail, 2017). This response can probably be explained by the action of PBZ resulting in the reduction of endogenous GA levels, which enhanced cell division and cell elongation.

There was no significant difference in leaf area among the PBZ concentrations of GE344 hybrid, however, GE341 and SSH348 hybrids showed significant difference across PBZ concentration. The highest concentration (1000 ppm) had 48 and 45% less leaf area than control at GE341 and SSH348 hybrids, respectively. Moreover, there was no significant difference among the watermelon hybrids regardless of PBZ concentration, where increasing concentration of PBZ reduced the leaf area of all tested hybridss. Plants treated with the PBZ had smaller leaf area as compared to the control plants, This supports the previous experiments with watermelon (Flores *et al.*, 2018), potato (Nuraini *et al.*, 2018), tomato (Pal *et al.*, 2016) and okra (Jyothsna *et al.*, 2022). The reduction in leaf area in treated plants was most likely caused by increased ABA content, which inhibits GA

Table 1. Plant growth of watermelon as a foliar application of paclobutrazol on hybrids

Treatment	Plant height (cm)	Internode length (cm)	Leaf area/plant (cm^2)
PBZ (ppm)			
0	417.35±1.13a	11.02±0.28a	5062.66±56.15a
250	357.84±2.53b	7.83±0.09b	4802.11±48.45b
500	278.21±1.30c	6.24±0.18c	3873.00±79.78c
750	256.70±1.24d	5.02±0.14d	3224.33±71.51d
1000	240.93±1.43e	5.06±0.20d	2838.66±93.83e
Hybrid (V)			
GE344	311.64±17.88a	7.30±0.65a	4177.33±53.93a
GE341	307.18±17.72b	6.79±0.54b	3729.66±62.73c
SSH348	311.80±18.22a	7.01±0.67ab	3973.46±62.60b
Significance level			
PBZ (ppm)	**	**	**
Hybrid (V)	*	*	**
PBZ × V	NS	NS	**

Different letters followed by figures differ significantly. NS–Not Significant.

biosynthesis (Liu and Hou, 2018). The two-way interaction between PBZ concentration and hybrid for leaf area/plant was highly significant (Table 2).

The hybrid GE344 treated with PBZ at the rate of 250 ppm exhibited a normal growth, while effects were more obvious at higher PBZ concentrations and caused huge reduction in leaf area/plant. The highest fresh (597.50 g) and dry (72.75 g) weight of shoot/plant was recorded at 250 ppm PBZ as compared to the lowest (466.34 and 44.90 g, respectively) at the highest (1000 ppm) concentration (Table 3). The hybrids GE344 and SSH348 showed near identical fresh (542.06 and 539.56 g), and dry shoot weight (61.02 and 61.13 g), respectively). Similar findings were reported by Araújo *et al.* (2020) in potato and Ahmad *et al.* (2015) in ornamental plants, treated with paclobutrazol. Shoot growth reduction occurred primarily due to decreased internode length (Table 1). Sofy *et al.* (2020) also reported reduced cell proliferation caused by the application of PBZ as responsible for restricted stem growth.

Fresh and dry weight of roots/plant was the maximum at 250 and 500 ppm, respectively with a respective increase of 42 and 56% than the control. Fresh weight of root/plant (8.88 to 9.79 g) did not differ significantly, however, dry weight was significant among hybrids. Root dry weight was statistically similar between GE344 and SSH348, which was reduced by 13 and 10% in GE341. Paclobutrazol application also significantly improved both the fresh and dry weight of roots, with the major increase occurring when 250 ppm PBZ was applied. These findings are in agreement with those reported by Salari *et al.* (2017) on olive, sprayed with PBZ. Root growth increased by PBZ, most probably due to an increase in endogenous levels of cytokinin (Tesfahun, 2018).

There was no significant difference in root length density among 250, 500, 750 and 1000 ppm of PBZ. Root length density was reduced

Table 2. Leaf area of watermelon as a foliar application of paclobutrazol on hybrids

PBZ concentration (ppm)	Leaf area (cm^2)/plant		
	GE344	GE341	SSH348
0	5179±43.67 aA	4957±31.18 aA	5098±50.24 aA
250	4919±7.75 aB	4613±6.85 bB	4873±9.78 aB
500	4127±11.48 aC	3583±10.90 cC	3908±4.95 bC
750	3471±7.42 aD	2984±16.46 cD	3217±8.09 bD
1000	3189±12.77 aE	2558±14.55 cE	2769±11.09 bE
Significance			
PBZ (ppm)		**	
Hybrid (V)		**	
PBZ × V		**	

Different letters followed by figures differ significantly.

Table 3. Shoot and root biomass of watermelon as a foliar application of paclobutrazol on hybrids

Treatment	Fresh weight of shoot/plant (g)	Dry weight of shoot/plant (g)	Fresh weight of root/plant (g)	Dry weight of root/plant (g)
PBZ (ppm)				
0	584.25±4.09b	72.49±0.65a	8.04±0.36b	0.78±0.06c
250	597.50±4.24a	72.75±0.71a	11.41±0.60a	1.02±0.07b
500	529.63±5.15c	60.55±0.98b	10.30±0.54a	1.22±0.16a
750	486.36±5.48d	49.26±0.66c	8.69±0.51b	0.94±0.16b
1000	466.34±4.66e	44.90±0.68d	8.81±0.36b	0.95±0.11b
Hybrid (V)				
GE344	542.06±13.95a	61.02±2.92a	9.68±0.40a	1.04±0.19a
GE341	516.82±14.47b	57.81±3.11b	8.88±0.49a	0.90±0.16b
SSH348	539.56±12.91a	61.13±3.25a	9.79±0.53a	1.00±0.18a
Significance level				
PBZ (ppm)	**	**	**	**
Hybrid (V)	**	**	ns	**
PBZ × V	NS	NS	NS	NS

NS–Not Significant. Different letters followed by figures. differ significantly.

by 34% at 0 ppm compared with 750 ppm (Table 4).

Among three hybrids, root length density ranged from 348.74 cm cm^{-3} in SSH348 to 303.70 cm/cm^3 at GE341. The results showed that PBZ treated plants had a higher root length density (RLD). This finding seems to be consistent with other researcher who observed that PBZ contributed in increasing RLD in maize (Kamran *et al.*, 2018). RLD was more effectively increased in 500 ppm of PBZ, however, no significant differences were observed when compared with other treatments except control. Roots with larger root length (RLD) and root surface area density (RSD) could provide more nutrients to the plant than smaller roots (Guan *et al.*, 2014). Additionally, triazoles were found to suppress gibberellic acid and increase the levels of endogenous cytokinin and ABA (Desta and Amare, 2021), which may be one of the possible causes of the improved root length in PBZ-treated plants.

Root diameter was the highest at 500 ppm of PBZ, which was statistically at par with 250 and 100 ppm of PBZ, but 58% higher than the control (Table 4). There was no effect of hybrid on root diameter of watermelon. Concentrations of PBZ were effective in increasing root diameter, however, the root diameter was decreased by the increasing of PBZ concentration from 750 to 1000 ppm. Kamran *et al.* (2018) noticed a similar increasing trend of root diameter with PBZ treated *Solanum tuberosum* L. and treated maize seedlings. According to Kamran *et al.* (2018), in response to PBZ application, the cortex's thickness increased and more secondary xylem vessels were induced, which resulted in an increase in root diameter. Root diameter had shown to have a significant influence on the ability of roots to penetrate into the soil (Lynch *et al.*, 2022), extract water and nutrient in deep soil layers and translocate to aerial parts of the plant. Additionally, larger cortical parenchyma was correlated with increases in root diameter or increases in the number of rows and diameter of cortical cells, as seen previously in PGR-treated maize (Kamran *et al.*, 2018).

Significantly the highest relative chlorophyll content was recorded from 500 ppm of PBZ, followed by 250, 1000 and 750 ppm as against the lowest value from control treatment. PBZ-treated plants had higher chlorophyll content in leaves (Table 4). Flores *et al.* (2018) also noticed an increment in chlorophyll content of watermelon and cucumber leaves as a result of PBZ treatment. The PBZ at 250 ppm in hybrid GE344 was more effective to other treatment. The higher relative chlorophyll content of treated watermelon leaves may be related to the influence of PBZ on endogenous cytokinin levels. It was proposed that PBZ boosted cytokinin synthesis which enhanced differentiation of the chloroplast, chlorophyll biosynthesis and prevented chlorophyll degradation (Soumya *et al.*, 2017).

The highest number of female flowers per plant was obtained in plants sprayed with 250 ppm

Table 4. Root growth, relative chlorophyll content and number of female flowers of watermelon as application of foliar PBZ on hybrids

Treatment	Root length density (cm/cm^3)	Root diameter (mm)	Relative chlorophill content (SPAD)	No. of female flowers
PBZ (ppm)				
0	247.81±24.39b	1.64±0.15b	54.39±0.84e	2.88±0.13c
250	344.02±14.53a	2.11±0.21ab	77.92±1.76b	4.96±0.27a
500	315.11±21.28a	2.59±0.23a	87.65±2.32a	4.25±0.17b
750	378.51±27.26a	1.79±0.09b	67.72±1.73d	4.33±0.20b
1000	317.02±11.37a	2.39±0.15a	73.84±1.30c	3.77±0.24b
Hybrid (V)				
GE344	309.04±21.42a	2.08±0.20a	77.01±3.45a	4.37±0.28
GE341	303.70±16.09a	2.18±0.09a	68.56±2.74c	3.84±0.20
SSH348	348.74±23.36a	2.05±0.17a	71.35±2.98b	3.90±0.20
Significance level				
PBZ (ppm)	**	**	**	**
Hybrid (V)	NS	NS	**	NS
PBZ × V	NS	NS	NS	NS

NS-Not Significant. Different letters followed by figures dipper significantly.

PBZ treatment indicating an increase of 72% as compared to non-treated plants. Compared to control, the number of female flowers for 500, 750 and 1000 ppm was increased by 47, 50 and 31%, respectively, though, no significant difference was observed among them. The number of female flowers per plant was lowest in the control treatment. However, the only marked increase was at 250 ppm and then there was a sharp decline with further increase of PBZ concentrations. The number of female flowers significantly increased with PBZ concentrations. Treated plants had high number of female flowers than the control plants. Gerdakaneh *et al.* (2018) showed similar effect of PBZ on pumpkin. These results indicated that GE344 hybrid treated at the rate of 250 ppm of PBZ was more effective in induction of female flower than other concentrations and hybrids. The anti-gibberellin effect of PBZ caused cessation of mitotic processes in the shoot meristem might influenced the number of female flowers (Gerdakaneh *et al.*, 2018).

There was no significant difference in average fruit weight between 0 and 250 ppm of PBZ. Average fruit weight was reduced by 20 and 37% at 750 and 100 ppm compared with 250 ppm PBZ (Table 5). Among the hybrids, GE344 resulted in the maximum fruit weight, which was statistically identical to SSH348 hybrid as compared to the minimum from GE341 hybrid. Plants treated with PBZ under low concentration slightly increased the fruit weight while with increasing PBZ concentration the weight was greatly decreased. GE344 hybrid treated with PBZ at the rate 250 ppm had good result than other two hybrids. Higher concentration of PBZ 750 and 1000 ppm had adversely affected the fruit size and weight which resulted in decreased yield for all three hybrids. Similar findings were reported by Kishore *et al.* (2019) in mango that under higher concentrations of PBZ the mean fruit weight decreased. A progressive decrease in fruit length with increasing concentration of PBZ was observed. As compared to 1000 ppm, this trait was increased by 31 and 23% at 0 and 250 ppm, respectively. Among three hybrids, fruit length was significantly higher at GE344 and SSH348 than GE341. Fruit diameter was the highest at 250 ppm as against the lowest from control plant. Fruit diameter ranged from 11.55 cm at GE341 to 12.29 cm at GE344 hybrid. Similar findings were reported by Bhutia *et al.* (2017) when PBZ was applied to litchi which resulted in an increment in the mean fruit diameter.

Fruit firmness was the highest at 250 ppm PBZ where it was reduced by 42% than control treatment. PBZ-treated plants produced greater firmness of fruits as compared to non-treated as reported by Vázquez-Luna *et al.* (2014) that application of PBZ (Cultar) in mango trees improved fruit quality, especially external fruit appearance and firmness. In contrast, in Fuji apple PBZ application had no significant effect on fruit firmness (Sha *et al.*, 2021).

The total soluble solids (TSS%) of 250, 500, 750 and 1000 ppm PBZ treatments were increased by 51, 25, 28 and 28%, respectively, compared to control plants, while there were no significant differences among 500, 750 and 1000 ppm PBZ treatment. The result also

Table 5. Fruit growth and fruit quality of watermelon as application of foliar paclobutrazol on hybrids

Treatment	Average fruit weight (kg)	Fruit length (cm)	Fruit diameter (cm)	Fruit firmness (N)	Total soluble solids (^{0}Brix)
PBZ (ppm)					
0	3.76±0.08a	20.63±0.54a	11.16±0.40c	2.88±0.13c	7.44±0.27c
250	3.64±0.11ab	19.32±0.80a	13.27±0.29a	4.96±0.27a	11.27±0.45a
500	3.57±0.09b	17.23±0.70b	11.72±0.22bc	4.25±0.17b	9.27±0.28b
750	2.88±0.07c	16.25±0.57b	12.02±0.23b	4.33±0.20b	9.52±0.36b
1000	2.27±0.09d	15.68±0.65b	11.71±0.22bc	3.77±0.24b	9.55±0.45b
Hybrid (V)					
GE344	3.42±0.15a	18.15±0.63a	12.29±0.41a	4.37±0.30	10.31±0.47a
GE341	2.95±0.15b	16.44±0.53b	11.55±0.22b	3.84±0.20	9.23±0.33b
SSH348	3.30±0.16a	18.88±0.77a	12.09±0.26ab	3.90±0.20	8.70±0.34b
Significance level					
PBZ (ppm)	**	**	**	**	**
Hybrid (V)	**	**	NS	NS	**
PBZ × V	NS	NS	NS	NS	NS

NS–Not Significant. Different letters followed by figures differ significantly.

indicated that the highest rate of TSS was obtained in 250 ppm and the lowest was for control plants. Among the hybrids, the highest mean values for TSS were recorded in GE344 (10.31 °Brix), followed by GE341 (9.23 °Brix) and SSH348 (8.70 °Brix), while there was no significant difference within GE341 and SSH348 (Table 3). The TSS% of fruits treated with PBZ, for all three tested hybrids, was significant more than untreated or control and 250 mg/l was more effective to other concentrations. Our results are in line with the results reported by Abed (2018) on the enhancement of total soluble solids in Lettuce treated with PBZ. TSS% was higher in hybrid GE344 than GE341 and SSH348 hybrids. It was speculated that paclobutrazol, while inducing growth restriction, may tend to reduce photo-assimilate demand of the growing shoot in favour of superfluous sinks (fruits). This was expected to increase fruit soluble solids, and a corresponding decrease in acidity.

CONCLUSION

Plant growth, number of female flowers and fruit growth including fruit quality were affected by the foliar application of PBZ. Application of PBZ was beneficial across all watermelon hybrids. Among the PBZ concentrations, 250 ppm was recommended to retard the vegetative growth of watermelon, induce number of female flowers/plant, enhance fruit growth and improve fruit quality inside greenhouse condition. Thus, PBZ at lower concentration was found effective in controlling the vigour of watermelon without markedly affecting fruit size compared with higher rate of PBZ. This study showed that the GE344 Mellow Oblong hybrid outperformed other hybrids in terms of relative chlorophyll content, TSS%, fruit weight, number of female flowers and root volume.

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