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Influence of paclobutrazol on growth, root traits, anatomical modifications and leaf nutrient status in mango

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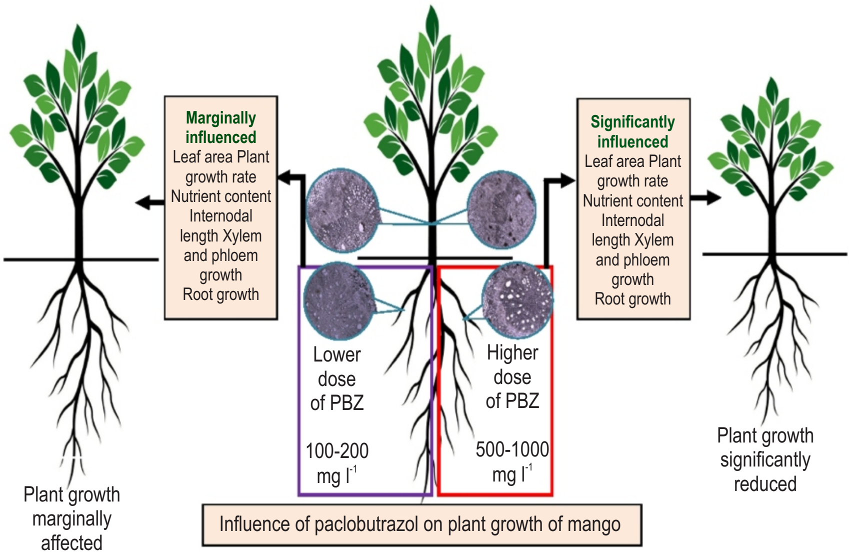
Abstract

Aim: Present study aimed to assess the efficacy of paclobutrazol on vegetative growth, root traits, antomical modifications and nutrient uptake in mango.

Methodology: Pot experiment was conducted in young grafted plants of Amrapali variety treated with four concentrations of paclobutrazol *viz.,* 100 mg l^{-1}, 200 mg l^{-1}, 500 mg l^{-1} and 1000 mg l^{-1}. The design was completely randomized with four replications and each replicate comprised of fifteen plants. Data on different parameters were recorded in randomly selected plants and mean values were presented.

Results: Plant height, leaf area, internodal length, plant biomass and shoot biomass reduced by 30.8%, 32.4%, 38.2%, 38.7% and 40.4%, respectively, at higher concentration of paclobutrazol (1000 mg l^{-1}). However, there was an increase in leaf chlorophyll content with high PBZ concentrations (1000 mg l^{-1}). Root traits like root biomass, growth rate and primary root reduced significantly at higher PBZ concentrations, whereas tertiary roots tended to increase. The size of xylem and phloem reduced substantially at higher PBZ concentration. First principal component (PC 1) explained more than 92% of the variance signifying most of the growth attributing traits.



Interpretation: The results unveiled that growth attributing traits, vascular characteristics of root and stem, and leaf nutrient status were significantly influenced by higher concentration of PBZ.

Key words: Anatomy, Leaf nutrient, Mango, Paclobutrazol, Plant growth, Root traits

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Introduction

Mango is the most important fruit crop of India which is cultivated in both tropical and subtropical climatic conditions. However, crop periodicity is strikingly evident in most of the leading commercial varieties of mango which is characterized with 'on' and 'off' year. During 'on' year flowering occurs profusely, whereas 'off' year signifies low intensity of floriferous shoots and dominance of vegetative shoots. The cyclic variation in yield may be primarily attributed to intrinsic factors and among them hormones, temperature and shoot age play critical roles (Remirez and Davenport, 2010). In mango, the fate of differentiating buds depends on the concentration of auxin and gibberellin. The former facilitates differentiation of reproductive buds, whereas latter promotes vegetative growth. The role of gibberellin in mango as a flower suppressor was corroborated when exogenous application of GA-inhibitor promoted flowering. Paclobutrazol (PBZ), a gibberellin inhibitor, exhibited its efficacy in regulating flowering in fruit crops. However, efficacy varies with climatic conditions, crop species, rate and methods of application (Nartvaranant *et al.,* 2000). PBZ is commonly used in mango to bring regularity in bearing through flower induction (Upreti *et al.,* 2013; Oliveira *et al.,* 2017).

Paclobutrazol is applied to plants through soil and foliar spray. However soil application around the tree trunk has been found more efficacious as it ensures proper uptake (Kulkarni *et al.,* 2006). The role of paclobutrazol in regulating root activity has been reported in different crops. Kotur (2006) observed significant variation in the root activity in paclobutrazol treated mango plants. Yamshita *et al.* (1997) also reported an increase in the content of fibrous roots in paclobutrazol-treated mandarin plants. Leaf nutrient content was also influenced by paclobutrazol. Arzani and Roosta (2004) observed reduction in leaf nitrogen content in PBZ-treated almond plant; however, P, K and Ca contents were unaffected. On the other hand, Arzani *et al.* (2009) reported that N and P were not influenced by PBZ treatments in peach. Kishore *et al.* (2016) observed inconsistent influence of paclobutrazol on leaf nutrient content as it varied with the crop species and soil conditions. Concerns have also been expressed over the use of paclobutrazol as it inhibits gibberellin bio-synthesis, which is responsible for cell elongation and internode extension. It has been reported that application of PBZ beyond its optimized dose causes stunting and produces compressed panicles in mango (Reddy and Kurian, 2008; Sharma and Awasthi, 2005).

It has also been reported that paclobutrazol causes anatomical modifications and alteration in root activity, and thereby influences nutrient uptake (Kurian and Iyer, 1992; Gomes *et al.,* 2012). In mango, the efficacy of PBZ has been assessed in growth, root activity and nutrient uptake by many researchers. However, most of the experiments have been carried out under field conditions wherein the exact impact of paclobutrazol on root activity is difficult to work out. To the best of our knowledge, anatomical modification in plant parts of mango has not been studied. Since the activity of root system of a plant significantly influences the above ground growth, a pot experiment was carried out to study the impact of PBZ on root traits, anatomical modifications, growth and nutrient composition of mango.

Materials and Methods

The experiment was conducted under open condition at the Central Horticultural Experiment Station, (ICAR-IIHR), Bhubaneswar during 2017-18. Four-month-old grafted Amrapali plants of uniform vigour were selected for pot experiment. Each pot contained 10 kg mixture prepared with soil, farm yard manure and vermicompost mixed in the proportion of 1:1:1. The pH, organic C, N, P and K content in plant growing medium was 5.7, 0.7%, 256 kg ha^{-1}, 24 kg ha^{-1} and 232 kg ha^{-1}, respectively. After a month, paclobutrazol was applied in soil in four doses *viz.* 100 mg l^{-1}, 200 mg l^{-1}, 500 mg l^{-1} and 1000 mg l^{-1}, whereas application of water was considered as a control. The doses of paclobutrazol were worked out on the basis of its molecular weight (293.79 g mol^{-1}) and concentration (23% w/w) in the synthetic formulation.

The design was completely randomized with five treatments each with four replications and each replicate comprised of fifteen plants. Soil moisture and pests was properly managed during the experiment. Data on plant growth such as plant height, shoot girth, intermodal length and leaf area were recorded after three and four months of treatment imposition in randomly selected five plants under each replication and mean value was worked out. Root characters and root/shoot ratio were studied by destructive methods and three plants were randomly selected under each replication after four months of treatment imposition. For absolute growth rate, initial and final dry weight of shoots and roots of randomly selected ten plants under different treatments were taken. Plant height was measured from the ground level to the growing tip with the help of a scale. The shoot girth and internodal length were measured a vernier calliper.

For anatomical studies thin cross sections of root, shoot and leaf were prepared and observed under microscope. The leaf area was computed on the basis of maximum length and maximum breadth of matured leaves of tagged seedlings and area was calculated using the formula of Ghoreishi *et al.* (2012). Plant biomass under different treatments was worked out at the beginning of experiment and after four months of treatment imposition. Under each treatment, ten plants were randomly selected from replicated plots and uprooted without any root damage. Plant parts (leaves, stem and root) were properly washed and dried in oven at 65°C for 72 hrs and dry weight was estimated. On the basis of average dry matter content in plant parts biomass was worked out. Plant growth of mango under different treatments was measured in terms of absolute growth rate (AGR) and expressed in g per day (Pandey *et al.,* 2017). Leaf chlorophyll content was measured following the method of Fan *et al.* (2013). For nutrient analysis, leaves were collected dried and grounded. Nitrogen was analysed by the Kjeldahl method. Potassium was quantified by flame photometry and phosphorus by molybdate method, Ca, Mg, Fe, and Zn were analysed using atomic absorption spectrophotometer. Data on plant biomass, growth, chlorophyll content and leaf nutrient content were analysed using One-way ANOVA. Duncan's Multiple Range Test was used to test statistical difference between means ($P < 0.05$) using OPSTAT statistical package. Principal component analysis (PCA) was performed on growth attributing traits including leaf nutrient status to explore relationships among variables and treatments. The first two principal components (PC1 and PC2) were selected for the analysis on the basis of cumulative variance (> 96%). This analysis was carried out using the software package XLSTAT.

Results and Discussion

The growth of mango grafts were influenced by paclobutrazol, however, intensity increased with the concentration (Fig. 1). At lower PBZ concentration (100-200 mg l^{-1}), reduction in plant height was less (6-10%), but higher concentration (1000 mg l^{-1}) induced substantial reduction in plant height (~ 30%). Similarly, shoot girth, intermodal length and leaf area were significantly affected by higher dose of PBZ. In comparison to untreated plant, shoot girth, internodal length and leaf area were reduced by 18%, 38.2% and 32.4%, respectively. However, these growth parameters were marginally reduced with lower concentration of paclobutrazol (Fig. 1). Kishore *et al.* (2019) also observed reduction in plant growth and leaf area with the application of paclobutrazol. When the impact of paclobutrazol on plant biomass was assessed, it was observed that at higher concentration (1000 mg l^{-1}) plant biomass and shoot biomass were significantly reduced by 38.7% and 40.4%.

It was also evident that the degree of reduction in the absolute growth rate of plant and shoot was proportionate to the concentration of paclobutrazol. The absolute growth rate of plant and shoot reduced approximately by 40% and 45% at higher concentration of paclobutrazol (1000 mg l^{-1}), whereas the absolute growth rate was marginally reduced at lower concentration. A similar trend was also observed in root biomass and absolute root growth rate as the former was reduced by 28% and latter by 31%, respectively (Fig. 2). Significant reduction in the vegetative growth and leaf area may be due to the reduction in endogenous gibberellin level influenced by paclobutrazol (gibberellins-inhibitor). Gibberellin regulates the natural developmental processes, including cell elongation and cell division by inducing transcription of genes involved in these processes, which culminate in stem growth in plants (Sun, 2010). Since GA stimulates elongation of cell and internode, PBZ-induced reduction in GA biosynthesis might have affected the shoot growth, leaf area and shoot girth (Wang and Irving, 2011).

The relative growth of shoot and root was ascertained by shoot/root ratio which was marginally influenced by paclobutrazol. At lower concentration of paclobutrazol, the shoot/root ratio was subtly reduced whereas the ratio was moderately reduced at higher concentration (500-1000 mg l^{-1}). It may be interpreted that under high concentration of paclobutrazol shoot biomass accumulation was relatively more affected than root biomass. Biomass partitioning among root components (primary, secondary and tertiary) was significantly affected by paclobutrazol. At higher paclobutrazol concentration, primary roots were significantly reduced (20%) whereas tertiary roots showed reverse trend and increased substantially (60%). Secondary roots were marginally impacted by paclobutrazol application (Fig. 2). Reduced concentration of GA in PBZ-treated plants might have reduced auxin and increased ABA content which could have affected root growth, shoot elongation and leaf expansion (Razem, *et al.,* 2006; Kou *et al.,* 2021).

Leaf chlorophyll content was subtly influenced when lower dose of PBZ was applied (Fig. 2). However, a perceptible increase in the chlorophyll content (~15%) was recorded when paclobutrazol was applied in higher concentration (1000 mg l^{-1}). Kumar *et al.* (2012) also observed an increase in the leaf chlorophyll content with the application of paclobutrazol has been observed in many crops. The increase in leaf chlorophyll content in PBZ-treated plants may be attributed to PBZ-induced synthesis of cytokinin which in turn enhances chloroplast differentiation and chlorophyll biosynthesis, and prevents chlorophyll degradation (Nivedithadevi *et al.,* 2015). The transverse sections of root, shoot and leaf of mango revealed anatomical modifications in PBZ-treated seedlings (Fig. 3). In mango roots, the xylem and phloem are radially arranged in separate bundles. It was observed that vascular bundles of root was unaffected with low concentration of paclobutrazol. On the other hand, the size of xylem and phloem

Table 1: Influence of paclobutrazol on radial thickness of vascular tissues of mango seedlings

PBZ concentration (mg l^{-1})	Root		Stem			Leaf
	Xylem (µm)	Phloem (µm)	Xylem (µm)	Phloem (µm)	Vascular bundle (µm)	Air space in spongy parenchyma (µm)
0	164.5^{a}	89.4^{a}	152.6^{a}	77.8^{a}	256.8^{a}	28.6^{a}
100	160.8^{b}	87.7^{b}	150.6^{a}	76.8^{a}	254.3^{a}	27.8^{a}
200	152.7^{c}	81.6^{c}	145.3^{b}	71.4^{b}	243.5^{b}	23.7^{b}
500	134.8^{d}	80.4^{c}	124.7^{c}	66.2^{c}	218.9^{c}	14.9^{c}
1000	129.7^{e}	71.8^{d}	112.9^{d}	64.9^{d}	204.6^{d}	8.3^{d}

Means followed by same superscripted letter(s) do not differ significantly ($P < 0.05$) by DMRT.

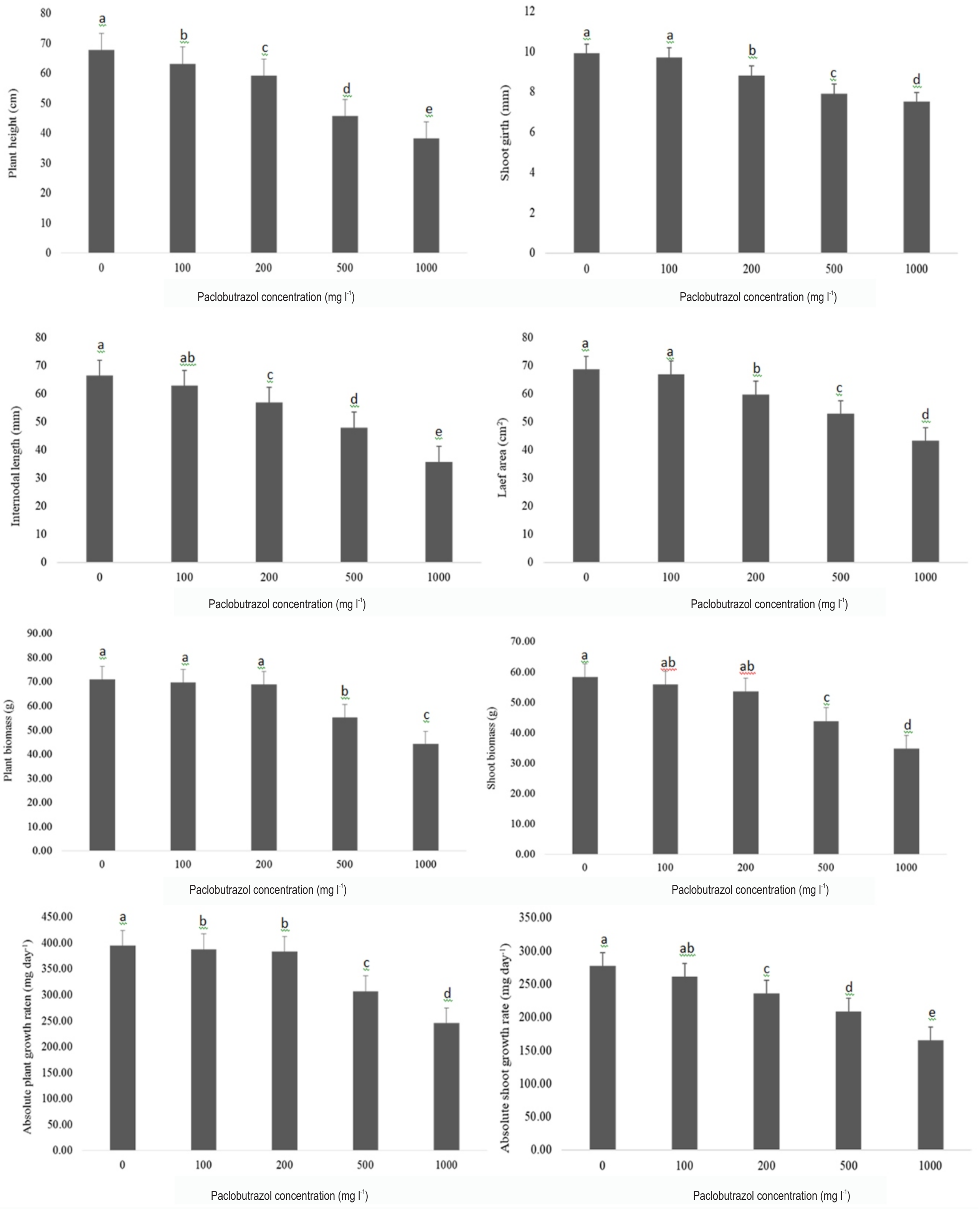


Fig. 1: Influence of paclobutrazol on growth attributes of mango. Data are mean values and bars show standard errors (±). Within a bar, different lowercase show significant difference ($P < 0.05$) between treatments.

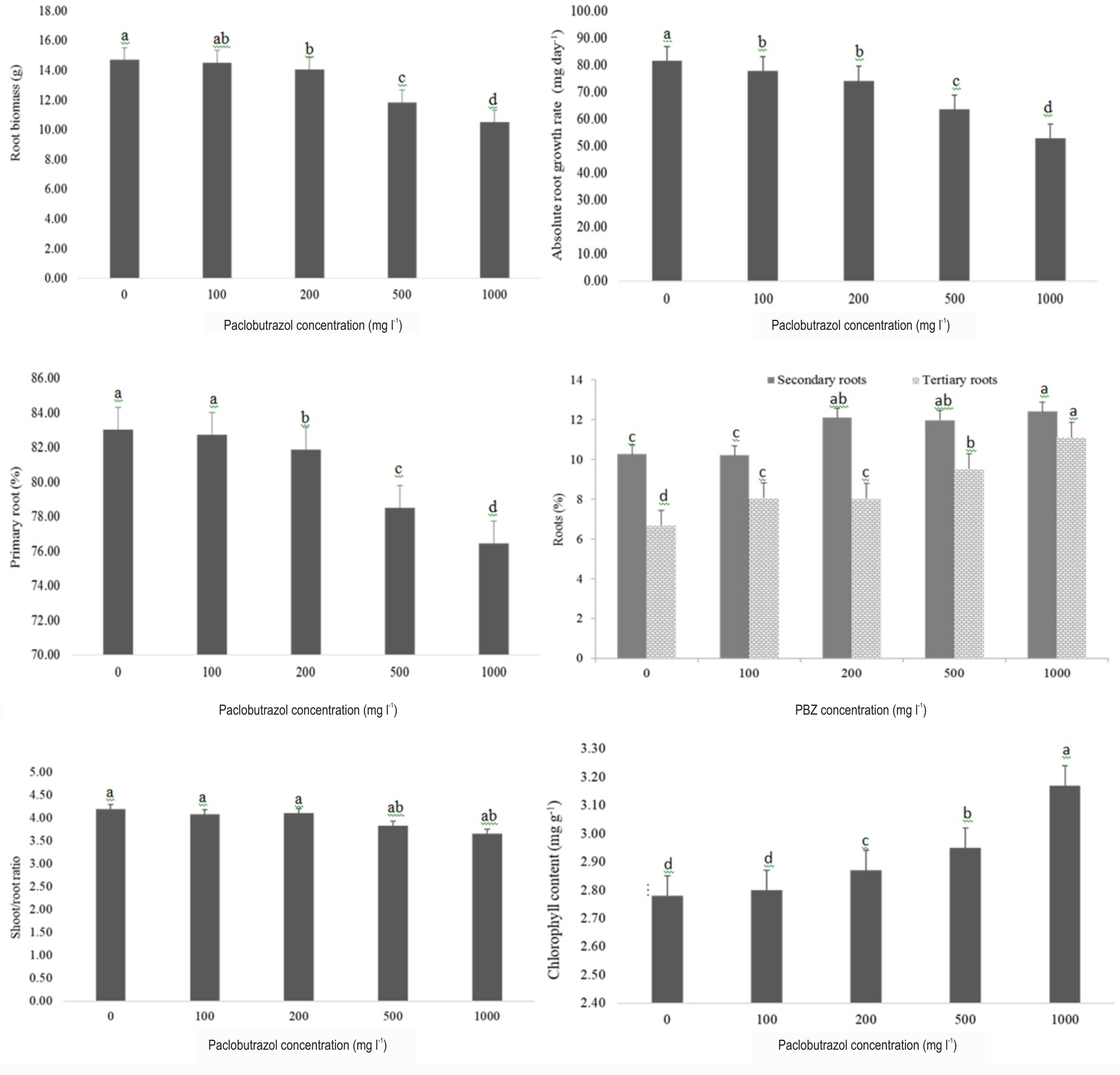
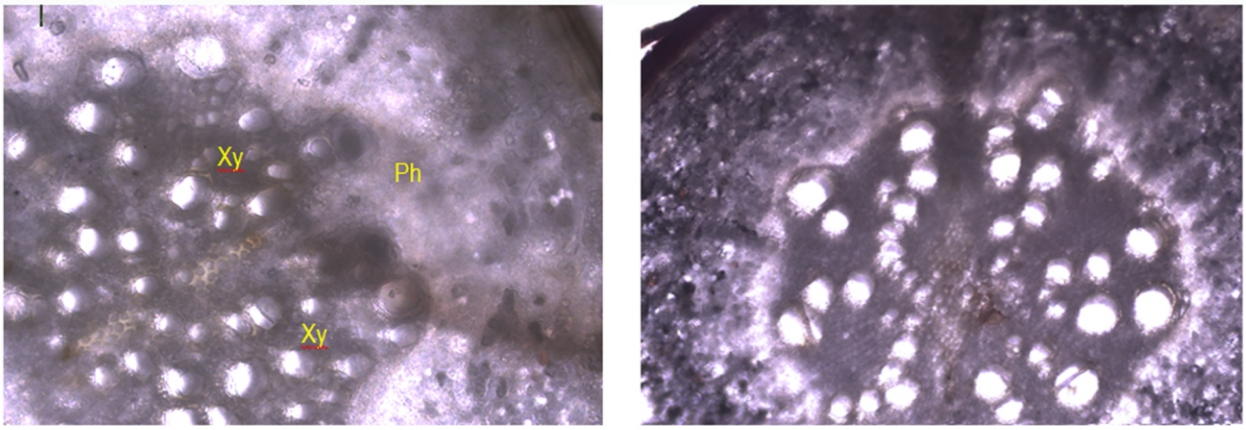


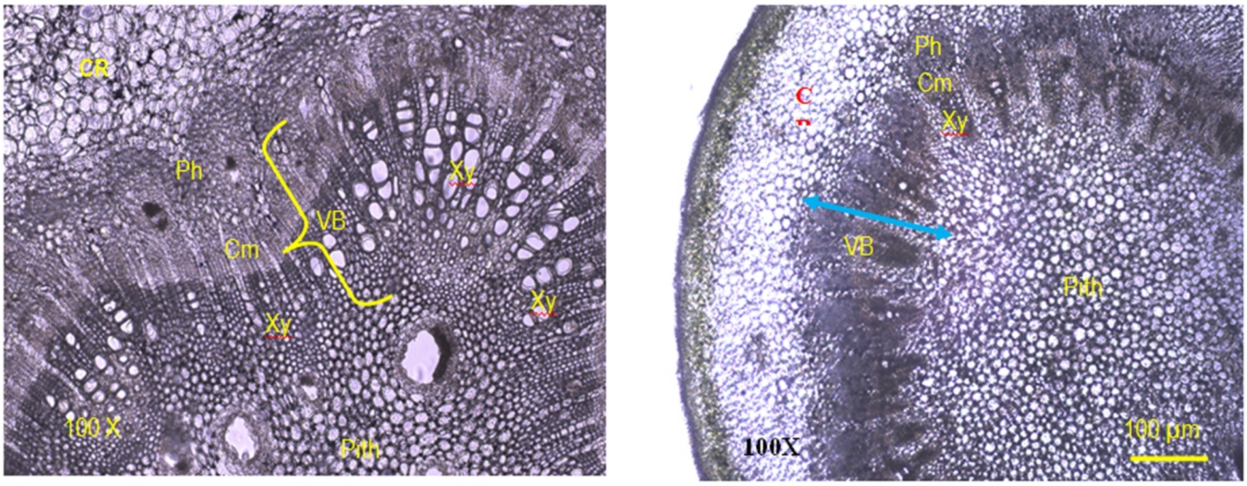
Fig. 2: Influence of paclobutrazol on root traits, shoot/root ratio and chlorophyll content of mango. Data are mean values and bars show standard errors (±). Within a bar, different lowercase show significant difference ($P < 0.05$) between treatments.

was reduced by 21.3% and 19.6%, when high concentration of paclobutrazol (1000 mg l^{-1}) was applied. It may be interpreted that the reduction in the size of xylem and phloem affected the absolute growth rate (AGR) of root as AGR was significantly correlated with the size of xylem (r = 0.953**) and phloem (r = 0.919**). The findings are in agreement with the report of Burrows *et al.* (1992), who observed inhibition of vascular development in the roots of chrysanthemum. Low concentration of paclobutrazol affected the size of VB imperceptibly, but high concentration of paclobutrazol (1000 mg l^{-1}) significantly affected the thickness of vascular bundle by reducing its size by 20.3% (Table 1). Moreover, the radial thickness of xylem and phloem was also reduced by 26.3% and 16.5% respectively. It may be deduced that the reduction in the size of vascular bundle affected absolute growth rate of shoot as the latter was significantly correlated with the size of former (r = 0.978**). Murti *et al.* (2001) also reported a reduction in xylem radial width in the shoots of paclobutrazol-treated mango tree, however, they observed increase in phloem width under paclobutrazol treatment. Rahman *et al.* (2016) observed smaller cortical cells in the stems of paclobutrazol-treated oil palm seedlings. Reduction in the size of vascular tissues in paclobutrazol-treated mango plants may be due to



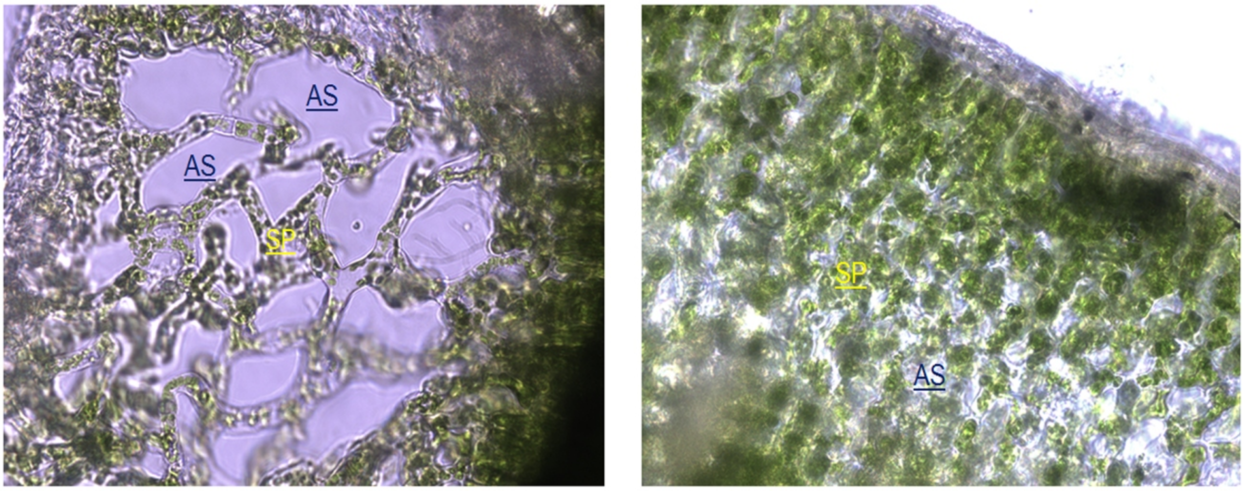
Cross section of untreated root

Cross section of PBZ treated root (5 µM)



Cross section of untreated shoot

Cross section of PBZ treated shoot (5 µM)



Cross section of untreated leaf

Cross section of PBZ treated leaf (5 µM)

Fig. 3: Microscopic view of TS of root, shoot and leaf of untreated and PBZ treated mango Xy – xylem; Ph – phloem; Cm – cambium; CR – cortical region; SP – spongy parenchyma; AS – Air space.

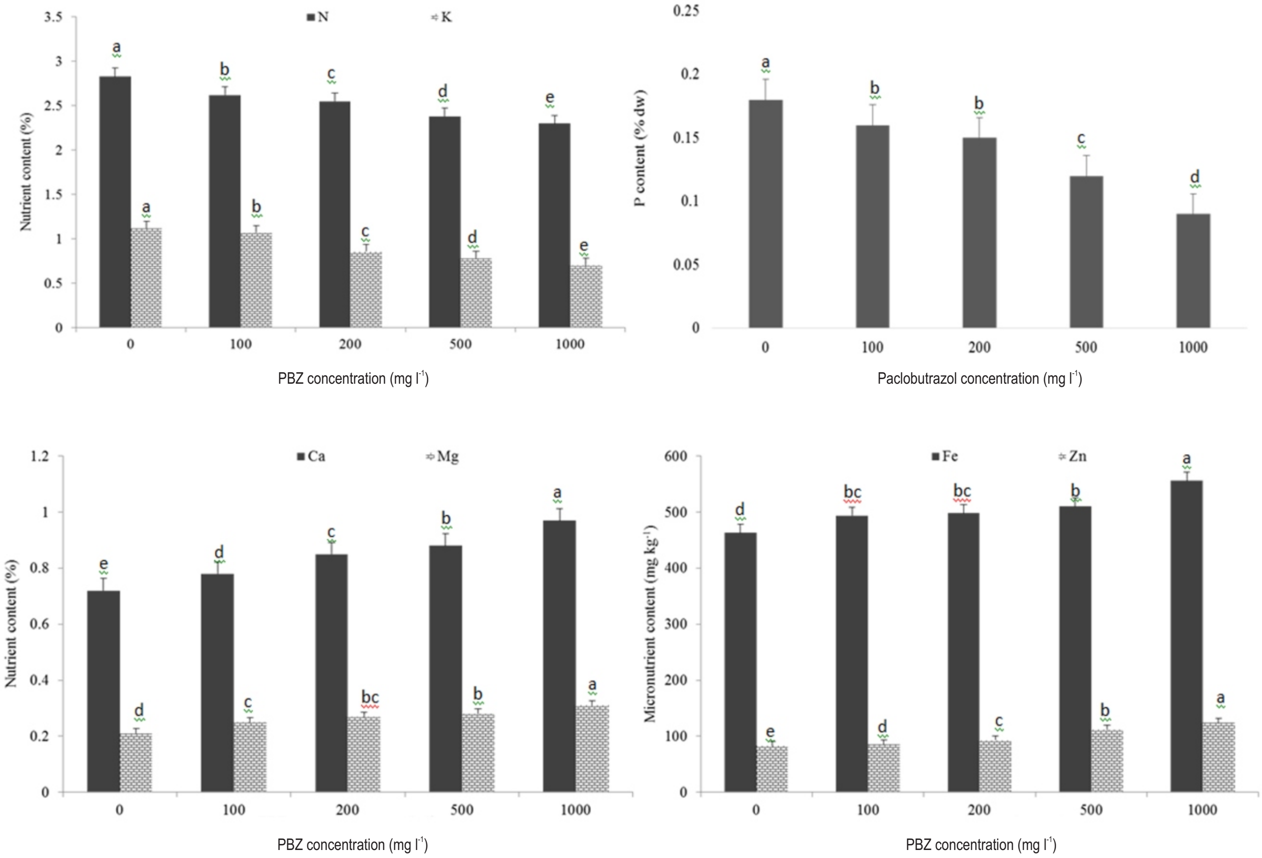


Fig. 4: Influence of paclobutrazol on leaf nutrient status of mango. Data are mean values and bars show standard errors (±).Within a bar, different lowercase show significant difference ($P < 0.05$) between treatments.

reduced synthesis of gibberellin which plays an important role in xylem differentiation, cambial activity and root elongation (Weiss and Ori, 2007; Aloni 2013). The leaves were also affected significantly by high paclobutrazol concentration (Fig. 3). The size of spongy parenchyma was significantly reduced which was evident with the 71.4% reduction in the size of air space (Table 1). Rodrigues *et al.* (2016) also reported an increase in thickness of leaf of *Toona ciliate* with paclobutrazol application. Leaf nutrient content was significantly influenced by paclobutrazol application, however, the degree of influence was related with the concentration of paclobutrazol (Fig. 4).

Higher concentration of paclobutrazol (1000 mg l^{-1}) substantially reduced the N (18.7%), P (46.8%) and K (27.4%) contents. On the other hand, the contents of Ca, Mg, Fe and Zn increased significantly at higher paclobutrazol concentration. Reduction in the leaf N and K contents in paclobutrazol-treated trees could be attributed to reduced root hydraulic conductivity and root volume, which in turn reduces water flux responsible for passive uptake of mobile nutrients like N and K (Reiger and Scalabrelli, 1990). The leaf Ca, Mg, Fe and Zn contents increased by 21.4%, 38.6%, 18.8% and 23.1%, respectively, when paclobutrazol was, applied @ 1000 mg l^{-1}. It was also evident that there was a marginal decrease in N, P and K contents and subtle increase in Ca, Mg, Fe and Zn contents in the leaves of mango when paclobutrazol was applied at a lower concentration ($\leq$ 200 mg l^{-1}). Kishore *et al.* (2019) observed a reduction in leaf N and K contents, and increase in the levels of Ca, Mg, and Zn when paclobutrazol was applied at a higher dose. It is evident that paclobutrazol increases Ca, Mg and micronutrients contents in leaves. However, the influence of paclobutrazol on N, P and K contents was inconsistent.

The whole data set was subjected to principal component analysis in order to obtain a comprehensive overview of variance of the plant growth traits in response to various concentrations of PBZ (Fig. 5). It is evident that the first principal component (PC 1) is associated with high eigenvalues by explaining more than 92% of the variance whereas PC 2 explained more than 4%. The cumulative variance explained by both the PCs was more than 96%. The loading plots of principal components illustrate the contribution of variables to principal components and the relationships among variables. It is evident that plant growth, absolute growth rate, plant biomass, leaf

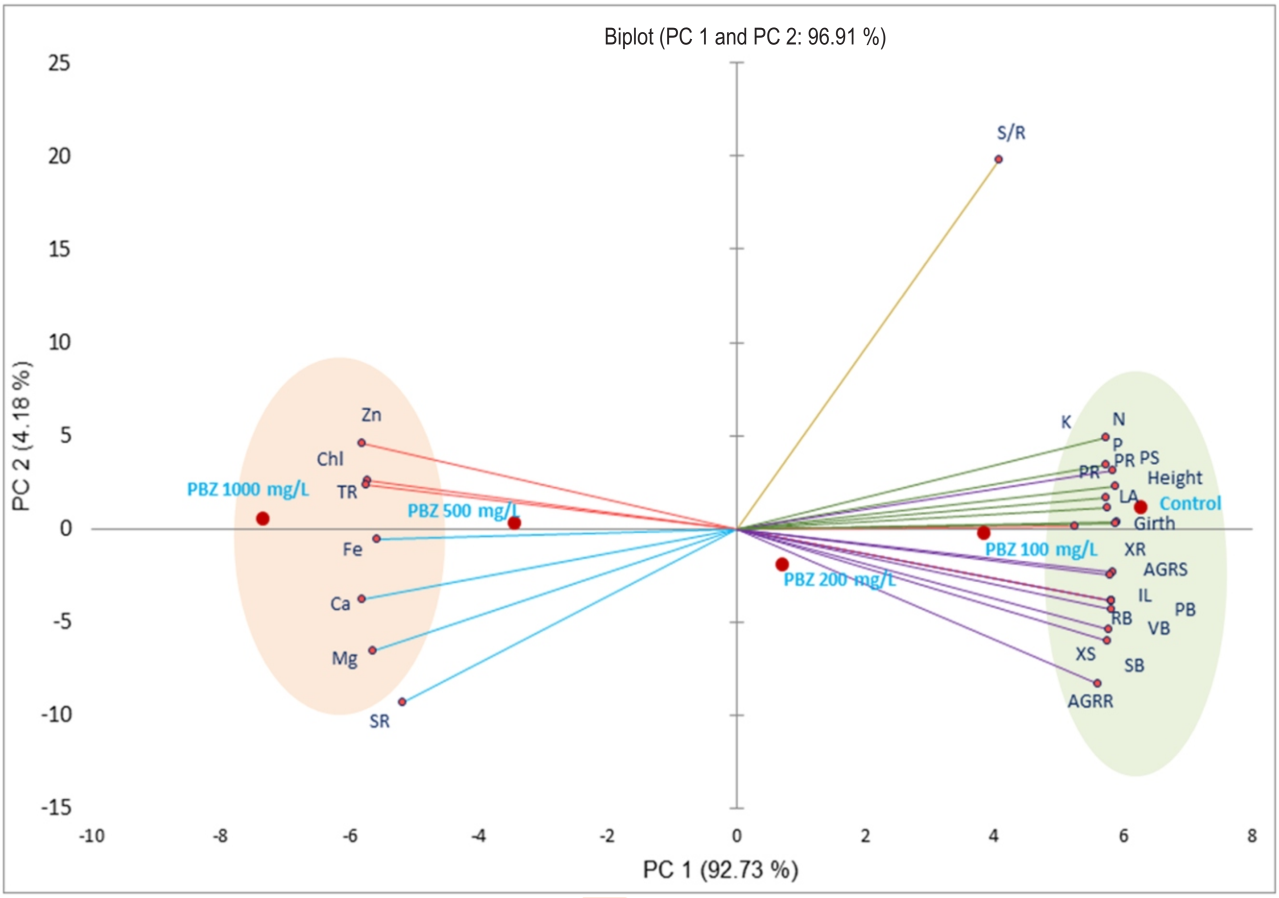


Fig. 5: Principal component loading plots for plant growth variables as a function of PBZ concentrations. N-nitrogen, P- phosphorous, K-potassium, Chl – chlorophyll, AGRS – absolute growth rate of shoot, AGRS - absolute growth rate of root, PR – primary root, LA – leaf area, VB- vascular bundle, XS- Xylem of shoot, XR – xylem of root, PR -phloem of root, PS-phloem of shoot, IL – internodal length, TR – tertiary root, RB-root biomass, SB- shoot biomass, S/R – shoot/root ratio, SR-secondary root.

nutrient content, leaf area, chlorophyll, internodal length, size of vascular bundle including xylem and phloem, primary root and tertiary root contribute significantly to PC1, hence the PC1 defines most of the growth attributing traits of treated mango plants (Fig. 5).

On the other hand, shoot/root ratio and secondary root contributed to PC 2. It is also apparent that plant growth, absolute growth rate, plant biomass, shoot biomass, root biomass, N, P, K, leaf area, internodal length, girth and size of vascular bundle were significantly and positively correlated whereas the leaf area was negatively correlated with Ca, Mg, Fe, Zn, chlorophyll content and tertiary root. Shoot/root ratio was not related with any of the growth-related traits. It was also evident that higher concentration of PBZ (500 mg l^{-1} and 1000 mg l^{-1}) significantly increased Ca, Mg, Fe, Zn, chlorophyll content and tertiary root of mango but significantly reduced plant biomass, growth rate, macronutrient, leaf area and internodal length. Paclobutrazol significantly influenced the growth of mango seedlings. It is evident that plant growth rate, leaf area, intermodal length and root traits were highly affected by higher concentration of paclobutrazol. Moreover, the size of xylem and phloem and leaf nutrient were highly influenced by higher concentration of paclobutrazol. The research findings will be useful in understanding the role of paclobutrazol in influencing the plant growth by regulating biomass accumulation, vascular differentiation and nutrient uptake. Since PBZ is commonly used in perennial fruit crops for ensuring regularity in bearing, higher dose might affect the growth, and in turn the yield potential of plant.

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Add-on Information

Authors' contribution: M.R. Sahoo: Carried out the experiment and recorded data; **K. Kishore:** Visualization, supervision of experiment, preparation and editing of manuscript; **D.K. Dash, C. M. Panda, R.K. Panda:** Provided critical feedback and contributed to the interpretation of the result; **P.K. Nayak:** Recording of data.

Research content: The research content is original and has not been submitted elsewhere for publication.

Ethical approval: Not applicable.

Conflict of interest: The authors declare that there is no conflict of interest.

Data from other sources: Not applicable.

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