



International Journal of Research in Agronomy

E-ISSN: 2618-0618
P-ISSN: 2618-060X
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NAAS Rating (2026): 5.20
www.agronomyjournals.com
2026; 9(1): 312-321
Received: 01-10-2025
Accepted: 18-11-2025

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Effect of stem tip pruning and bioregulators on endogenous Absciscic Acid (ABA), Cytokinins (DHZR'S and (ZR's), Gibberellic acid (GA₃), Auxin (IAA) contents at flower bud swelling stage (510 Stage OF BBCH Scale) in Mango (*Mangifera indica* L) cv. Banganpalli under high density planting system

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DOI: <https://www.doi.org/10.33545/2618060X.2026.v9.i1e.4657>

Abstract

An experiment on the effect of bio regulators (B₁ -Gibberilic acid GA₃ @ 50 ppm, B₂ -6 Benzyl aminopurine 6 BA @ 200 ppm, B₃ -Paclobutrazol PBZ @ 1500 ppm, B₄ -Naphthaleine acetic acid NAA @ 100 ppm, B₅ -PBZ @ 1500 ppm+BA @ 200 ppm, B₆ -PBZ @ 1500 ppm+ NAA @ 100 ppm, B₇ -GA₃ @.50 ppm+ PBZ @ 1500 ppm, B₈ - Untreated Control) and stem tip pruning (P₁ -with stem tip pruning, P₂ - without stem tip pruning) was conducted on endogenous absciscic acid (ABA), auxin (IAA), cytokinins (DHZR'S and (ZR'S), gibberellic acid (GA₃), at flower bud swelling stage (510 stage of BBCH scale) in mango cv. Banganpalli under high density planting system. Paclobutrazol in combination with 6 benzyl aminopurine (6-BA) (B₅) along with stem tip pruning (P₁) has significantly resulted in highest endogenous ABA, cytokinins, lowest GA₃ contents at flower bud swelling stage (510 stage of BBCH scale).

Keywords: Hormones in mango buds Benzylaminopurine, ABA, GA₃, ZR and DHZR, stem tip pruning, combination of stem tip pruning and bioregulators

Introduction

Mango (*Mangifera indica*. L) is the premier fruit among the tropical fruits and has been in cultivation in the Indian subcontinent since several centuries. Mango occupied an area of 2.26 million hectares with a production of 21.82 million tonnes. The fruit is highly valued because of its excellent flavor, appealing aroma, delicious taste, attractive shades of colour and nutritive value, which has attracted the world market. In Telangana, mango occupies an area of 0.18 million hectares with a production of 1.68 million tonnes. In Telangana state the commercial cultivar is Banganpalli which occupies about 70 per cent of total mango cultivated area. Of late, the production and productivity of mango cv. Banganpalli has been decreased in the past 4-5 years in Telangana. Productivity of Telangana state is 9.31 MT/ha which is very low when compared to mango growing states i.e., Uttar Pradesh (17.14 MT/ha), Andhra Pradesh (12.05 MT/ha), Karnataka (9.61 MT/ha), Bihar (16.37 MT/ha), Rajasthan (17.58 MT/ha). Poor productivity in mango cv. Banganpalli in Telangana is influenced by several factors such as improper pruning, delayed vegetative growth, poor and erratic flowering coupled with poor fruit set. However, there is tremendous scope to boost the productivity, if this problem can be managed properly.

The flowering phenomenon in mango appears to be a complex one. It is the key developmental event for crop yield and production. The intensity and timing of flowering shows strong dependence on physiological status of growing buds, hormonal interactions, environmental factors and nutrient availability. In mango, flowering is a complex process that involves differentiation of apical buds under the influence of low temperature and/or attaining of certain degree of shoot maturity followed by bud burst and panicle emergence (Davenport, 2007) [7].

Floral induction is considered to be the result of elevated levels of up-regulated florigenic promoter (FP) and down-regulated vegetative promoter (VP), primarily gibberellins, whereas the reverse condition promotes vegetative growth (Nartvaranant *et al.* 2000 and Davenport, 2007) ^[12, 7]. The production of vegetative shoots in place of reproductive shoots is due to the elevated level of gibberellin which is considered as a vegetative promoter. Over the past few years, disturbances in low temperatures, changes in the levels of florigenic promoter (FP) and vegetative promoter (VP) in the buds due to late and erratic rainfalls, which in turn lead to the extended period of vegetative growth phase over reproductive phase are the major factors that are responsible for the late, recurrent flowering in mango in Telangana.

The practice of special pruning technique, tip pruning in productive branches prevents continuous vegetative growth, which is a consequence of inappropriate climate conditions and has allowed for the high rate of flowering in axillary buds (Oliveira *et al.* 2015) ^[14]. Tip pruning is defined as pruning terminal stems anywhere from the apex to a point down stem that is no longer than 1 cm in diameter. This kind of pruning is done to assist in uniform flowering response in a flowering management program and to improve the productivity of bearing trees by significantly increasing the number of bearing stems (Davenport 2006) ^[6]. Tip pruning reduces the auxin synthesis at the apex of branches directing the transport of assimilates and cytokinins to the axillary buds of branches under flowering condition, inducing the formation of axillary inflorescences.

Various bio regulators application have been studied for enhancing uniform growth, flowering in mango. Foliar spray with GA₃ @ 100 ppm with severe pruning resulted in highest number of new flushes per shoots in zebda mango trees (Shaban, 2009) ^[21]. Application of 200 ppm of 6- benzyl amino purine significantly caused early flower induction, recorded highest flowering percentage, highest pure panicle percentage, highest yield over control in mango cv. Kesar (Shankar Swamy and Neelavathi, 2016) ^[23]. Naphthalene Acetic Acid (NAA) @ 80 ppm spray at 30 days before flowering was found to improve flowering in mango (Davenport, 2007) ^[7]. Plant growth retardants like paclobutrazol controlled tree growth and significantly increased number of panicles per shoot, fruit set and improved fruit quality (Vijay Krishna *et al.* 2020) ^[27].

Knowledge of the periodic biological events of a particular crop - bud breaks, flushing, flowering, and fruit development is an important tool for its agronomical management in relation with climate change (Hernandez Delgado *et al.*, 2011) ^[9]. Three digits "extended BBCH-scale" (Hack *et al.*, 1992) ^[8] was proposed for certain crops like grains, rape and sunflower, vegetables, pome and stone fruits, citrus, grape, strawberry, pomegranate, coffee, olive, Musaceae, persimmon, cherimoya. Keeping above fact in view, the phenological studies were conducted to assess BBCH-scale for mango cultivars like Totapuri (Shailender Rajan *et al.*, 2011) ^[22]. The BBCH scale distinctly separates the various vegetative flushes occurring in the mango, as well as the terminal and axillary flowerings. All of which is important for the correct timing of general orchard management, particularly for disease and pest management, physiological disorders and weed control, flowering inhibition and effectiveness of fertilizers and PGRs application (Hernandez Delgado *et al.*, 2011) ^[9].

Materials and Methods

The experiment was conducted on ten years old well grown, uniform statured trees of mango cv. Banganpalli. Trees are spaced with 5m and planted in square system. The statistical

design adopted was factorial randomised block design with 16 treatments which were replicated thrice. 200 mg of NAA was dissolved in 50 ml of ethanol and diluted it in 1 litre of water to get 200 ppm of NAA. 200 mg of 6-BA was dissolved in 50 ml of hydroxy acetone and diluted it in 1 litre of water to get 200 ppm of 6-BA. 50 mg of GA₃ was dissolved in 50 ml of ethanol and diluted it in 1 litre of water to get 50 ppm of GA₃. 1500 mg of paclobutrazol was diluted it in 1 litre of water to get 1500 ppm of paclobutrazol. Ten grams of Potassium nitrate (KNO₃) was dissolved in 1 litre of water to get 1% of KNO₃. 10 litres solution of each treatment was sprayed per tree uniformly at the time of application.

The above bioregulators were sprayed with and without stem tip pruning to observe the endogenous abscisic acid (ABA), auxin (IAA), cytokinins (DHZR'S and ZR'S), gibberellic acid (GA₃) at flower bud swelling stage (510 stage of BBCH scale).

The standard procedure for hormonal analysis during flower bud differentiation as per (Upreti *et al.*, 2013) ^[26] was followed.

Hormones analyzed

1. Absciscic acid (ABA)
2. Gibberellins (GA₃)
3. Cytokinins (ZR&DHZR) and
4. Auxins (IAA)

Type of samples

1. Varieties showing normal and off -season flowering
2. Off season flowering induction by chemical means

Stages of sampling

Flower bud differentiation and panicle emergence samples are taken from:

1. 3rd and 4th leaves from matured shoots and apical buds from those shoots.
2. Shoots radical diameter in a variety is taken as index for shoots of similar maturity.
3. Broadened & swollen buds to identify differentiating buds and bursting buds.

Tissues to be taken

Whole shoots with leaves & Buds - leaves and buds separated and used for sample preparation.

Amount of sample taken

5 grams leaves and buds are weighted accurately in an analytical balance.

Samples are collected in ice box

Leaf and buds samples were washed in water and air dried. The samples were crushed by putting liquid nitrogen using quartz sand using pestle and mortar. 25 ml of 80% cold methanol containing 0.25% BHT added to crushed samples. Shaken well after adding extraction solvent. Kept overnight, filtered, residue again taken-up in the same solvent and kept overnight again. Filtered again and combined with previous filtrate. Pooled methanolic sample is stored.

Sample storage

50 ml screw capped glass tubes in refrigerators at 4°C. Tubes were protected from exposures to sun florescent light.

Precautions taken

1. The samples are crushed and put in solvent on the same day of sampling.
2. The extraction procedure was carried out under diffused light.

3. To minimize contamination of plant tissue gloves were used during extraction.
4. All the solutions were well in advance cooled before starting.
5. Mortar & pestle were washed each time for different samples.
6. Hand gloves were used while transferring liquid nitrogen.
7. Samples were brought from field in ice box.

The samples were analyzed at IIHR, Bangalore for hormones through HPLC (High performing liquid chromatography).

a. ABA and cytokinin analysis

The bud and leaf samples (5 g fresh weight) were macerated in chilled 80% methanol and filtered. The filtrate was evaporated in vacuum at 35 °C, the residue was dissolved in water and pH was adjusted to 3.0. The aqueous acidic extract was partitioned twice against equal volumes of chilled diethyl ether. The ether phase was separated and dried over anhydrous sodium sulphate. The extract was kept overnight at 4 °C, filtered and after evaporating ether, the residue was dissolved in 20 mM Tris buffer, pH 7.8 for the ABA analysis. For the cytokinin analysis, the pH of aqueous extract left after ether partitioning was adjusted to pH 8.0 and partitioned thrice with equal volumes of water-saturated n-butanol. The butanol phase was separated, evaporated in vacuum at 35 °C and the residue was dissolved in Tris buffer (20 mM, pH 7.8) for cytokinin analysis. The ABA (Weiler) and the cytokinins - ZR and DHZR (Barthe and Stewart) contents were determined by ELISA using ELISA reader (Multiskan MS 352, LabSystem, Finland), employing laboratory raised polyclonal antibodies.

b. Gibberellins analysis

Gibberellins assay was done using gas chromatography mass spectrometry (GC-MS). Samples for gibberellin analysis using SIM (selected ion monitoring) were prepared according to Kaufman *et al.* (1976). Samples of apical buds (5 g) were separately grounded in liquid nitrogen and extracted thrice with chilled 80% aqueous methanol (8 ml g⁻¹). Pooled filtrates were dried in vacuum, dissolved in 10 ml of water and pH adjusted to 3.0. The aqueous acidic extract was partitioned against ethyl acetate three times, and ethyl acetate phase after separation was evaporated, re-dissolved in 0.1 M phosphate buffer, pH 8.0 and stirred with 2 g of insoluble polyvinylpyrrolidone. After filtration, pH was lowered to 3.0 and acid extract was partitioned thrice with ethyl acetate. After evaporating ethyl acetate, the gibberellin samples were purified on charcoal-elite (1:2 w/w, 5 g charcoal) adsorption column and silicic acid 100 mesh partition columns. Gibberellins were eluted from charcoal-celite column employing 80% acetone and gradiently eluted using 30-65% ethyl acetate in n-hexane from silicic column. The purified gibberellins were passed through 0.45 µm millipore filter (Fluoropore). For gibberellins assay by GC-MS, the samples were first methylated and further derivatized to trimethylsilyl (TMS) ether with 50 ml of bis-(trimethylsilyl) trifluoroacetamide (BSTFA), 1% trimethylchlorosilane (TMCS) at 60 °C for 1 hour. The derivatized samples were analysed on GC-MS system (Varian, USA) equipped with 3800 series gas chromatograph and 4000 series ion trap mass spectrometer. A VH-5 column (100-200 mesh, 30 m 9 0.2 mm id) programmed at 100 °C for 7 min, followed by a linear increase @ 10 up to 200 °C with 3 min hold and @ 5 °C with 19 min hold (total run time of 55 min) was employed to resolve silylated gibberellins. The split ratio was 1:10. The helium gas inlet was pneumatic pressure controlled at

a constant flow of 1 ml min⁻¹ and injector, transfer line, source and trap temperatures were maintained at 285, 270, 210 and 200 °C, respectively. The data were obtained in the full scan mode. Identification was confirmed on the basis of retention time and co-occurrence of additional ions employing NIST-2007 library. The quantification was done on the basis of peak area and the standard gibberellins (Sigma-Aldrich, USA).

c. Indole acetic acid analysis

Indole acetic acid (IAA) was assayed by Salkowsky's colorimetric method. The presence of IAA in samples was analyzed by thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC) using technical grade IAA as standard. Thin layer chromatography chromatograms were run on thin silica gel plates. Solvent system used to separate indole compounds was ethyl acetate: butanol: ethanol: water [5:3:1:1 (v/v)]. Indole acetic acid was detected on TLC plates by spraying with Kovac's reagent. Indole acetic acid assay was done by injecting 20 µl of the filtered extract onto a five µm reverse phase column (Waters Associates IBondapak C18, 250 mm 9 4 mm) in a waters associates HPLC equipped with an ultraviolet detector absorbing at 208 nm. The solvent system used to separate indole compounds was water: acetonitrile [76:24 (v/v)], and the flow rate was maintained at 1 ml min⁻¹. Quantification was done by comparison of peak heights.

Results and Discussion

1. Absciscic Acid (ABA) (ng /g)

The results on endogenous absciscic acid (ABA) (ng /g) concentration after stem tip pruning and different bioregulators are presented in the Table 1 and Fig 1. The pooled data revealed significant difference among different bioregulators with respect to absciscic acid concentration (ABA) (ng /g) at flower bud swelling stage of mango. Among different bioregulators, maximum ABA concentration was recorded with application of paclobutrazol in combination with 6-Benzyl amino purine (6-BA) (B₅) (90.99 ng/g). Least quantity of ABA was recorded with gibberellic acid (B₁) (66.23 ng/g). Similar increase in ABA content due to paclobutrazol is reported by Vijaykrishna *et al.* (2020) ^[28] in mango cv. Banganpalli, Burondkar *et al.* (2016) in mango cv. Alphonso, Upreti *et al.* (2013) ^[26] in mango cv. Totapuri. Paclobutrazol besides affecting gibberellins also increases ABA and cytokinin contents concomitant with C:N ratio in mango buds to elicit flowering responses (Upreti *et al.* 2013). The role of high ABA content in the induction of floral bud formation has earlier reported by Chacko, (1986) ^[8] in mango. Paclobutrazol induced increase in ABA content in the present investigation is in agreement with the earlier reports of induction in ABA biosynthesis as a result of modifications in the isoprenoid pathway which is partially common in ABA and gibberellins biosyntheses (Murti and Upreti, 2000) ^[26]. The high ABA levels are expected to induce bud dormancy, which consequently helped in floral bud formation as flowering in mango is found to occur on buds at rest (Upreti *et al.* 2013) ^[26]. Increase in ABA content with 6-BA might be due to altered isoprenoid pathway due to elevated levels of cytokinins as 6-BA is a synthetic cytokinin and due to positive correlation between ABA and cytokinins.

Stem tip pruning significantly influenced absciscic acid concentration (ABA) (ng/g) of mango. Maximum ABA concentration was recorded with stem tip pruning (P₁) (82.17 ng/g) compared to without stem tip pruning (P₂) which has recorded minimum ABA concentration (P₂) (79.70 ng/g).

Increase in the ABA content with stem tip pruning (P₁) has resulted increase in per cent flowering (Table 4.1.6) in the present investigation. Similarly, higher levels of ABA content in mango cv. Amrapali at flower bud swelling stage with moderate pruning was earlier reported by Sanjay. Tip pruning reduces the auxin synthesis at the apex of branches directing the transport of assimilates and cytokinins to the axillary buds of branches under flowering condition, inducing the formation of axillary inflorescences. Pruning of mango trees is vital for regulating tree size, early flowering and advancing fruit harvest in mango and such beneficial effects were mediated through increases in ABA and decreases in GA₃ contents.

Significant differences were observed in the interaction effect between stem tip pruning and bioregulators with respect to abscisic acid concentration (ABA) (ng/g). Maximum ABA concentration was recorded with the application of paclobutrazol in combination with 6 benzyl amino purine (6-BA) (B₅) with stem tip pruning (P₁) (91.84 ng/g). Minimum ABA concentration was recorded with application of gibberellic acid (B₁) without stem tip pruning (P₂) (65.15 ng/g). The combined effect of paclobutrazol in combination with 6 benzyl amino purine and stem tip pruning might have resulted in increase in ABA content in the present investigation with respect to the interaction effect. Higher level of ABA is conducive to the process of flowering in mango and its manipulation by spray of synthetic substance or by pruning at appropriate time may be helpful to induce flowering during off season.

2. Dihydro Zeatin Riboside DHZR (Pg/g)

The results on endogenous Dihydro Zeatin Riboside DHZR (Pg/g) concentration after stem tip pruning and different bioregulators are presented in the Table 2 and Fig 2. The pooled data revealed significant difference among bioregulators with respect to Dihydro Zeatin Riboside DHZR (Pg/g) concentration at flower bud swelling stage of mango.

Maximum DHZR (Pg/g) concentration was recorded with the application of paclobutrazol in combination with 6 Benzyl amino purine (6-BA) (B₅) (301.79 Pg/g). Minimum quantity of DHZR (Pg/g) was recorded with gibberellic acid (B₁) (262.93 Pg/g).

Stem tip pruning significantly influenced DHZR (Pg/g) concentration of mango. Maximum DHZR (Pg/g) concentration was recorded with stem tip pruning (P₁) (290.58 Pg/g) compared to without stem tip pruning (P₂) which recorded minimum DHZR concentration (P₂) (288.31 Pg/g).

Significant differences were observed in the interaction effect between stem tip pruning and bioregulators with respect to Dihydro Zeatin Ribose DHZR (Pg/g) content. Maximum DHZR (Pg/g) concentration was recorded with application of paclobutrazol in combination with 6 benzyl amino purine (6-BA) (B₅) with stem tip pruning (P₁) (302.92 Pg/g). Minimum DHZR (Pg/g) concentration was recorded with application of gibberellic acid (B₁) without stem tip pruning (P₂) (260.82 Pg/g).

3. Zeatin Riboside (ZR) (pg/g)

The results on endogenous Zeatin Riboside (ZR) (pg/g) concentration with stem tip pruning and different bioregulators are presented in the Table 3 and Fig 3. The pooled data revealed significant difference among bioregulators with respect to Zeatin Ribose ZR (Pg/g) concentration at flower bud swelling stage of mango.

Maximum ZR (Pg/g) concentration was recorded with application of paclobutrazol in combination with 6 benzyl amino

purine (6-BA) (B₅) (275.48 Pg/g). Minimum quantity of ZR (Pg/g) was recorded with control (B₈) (231.15 Pg/g) which was on par with the application of gibberellic acid (B₁) (231.33 Pg/g).

Stem tip pruning significantly influenced ZR (Pg/g) concentration of mango. Maximum ZR (Pg/g) concentration was recorded with stem tip pruning (P₁) (257.69 Pg/g) compared to without stem tip pruning (P₂) which recorded minimum (255.01 Pg/g).

Significant differences were observed in the interaction effect between stem tip pruning and bioregulators with respect to Zeatin Riboside ZR (Pg/g) content. Maximum ZR (Pg/g) concentration was recorded with application of paclobutrazol in combination with 6 benzyl amino purine (6-BA) (B₅) with stem tip pruning (P₁) (276.63 Pg/g). Minimum ZR (Pg/g) concentration was recorded in control (B₈) without stem tip pruning (P₂) (227.48 Pg/g) which was on par with the application of gibberellic acid (B₁) without stem tip pruning (P₂) (230.47 Pg/g).

Maximum endogenous cytokinins (ZR's and DHZR's) were recorded with paclobutrazol in combination with 6 benzyl amino purine (6-BA) (B₅) with stem tip pruning (P₁) application. Abdel Rahim and Chen (1987) ^[4] in mango reported similar increase in endogenous cytokinins at floral bud formation. Similar increase in endogenous cytokinins (ZR and DHZR) due to 6-BA was reported by Chen (1987) ^[4], stating that elevated cytokinin levels found prior to flowering due to the application of 6-BA caused precocious bud break and increase in per cent flowering confirming that cytokinins are involved in stimulation of bud break. 6-BA, being a synthetic cytokinin stimulates protein synthesis and participates in cell cycle control in a cell division (George) might have increased the endogenous cytokinins (ZR's, DHZR's) in the present investigation. Similar increase in ZR's and DHZR's during flower bud swelling stage results were reported by Naphrom in mango cv. Tommy atkins. Similar increase in ZR's and DHZR's during flower bud swelling stage with paclobutrazol was reported by Upreti *et al.* (2013) ^[26] in mango cv. Totapuri, Vijaykrishna *et al.* (2020) ^[28] in mango cv. Banganpalli. The increase in cytokinins by Paclobutrazol could be the either due to stimulated synthesis of cytokinins that are transported to shoots or arresting of cytokinin degradation and is suggested to act positively in floral bud induction by regulating cell division process, as cytokinins are well accepted stimulators of cell division. Cytokinin molecule is obtained from isoprenoid pathway leading to gibberellin biosynthesis, thus paclobutrazol while blocking gibberellin biosynthesis would channelize the metabolic intermediates to cytokinin production (ZR's and DHZR's) (Upreti *et al.* 2013) ^[26]. Increase in ZR's and DHZR's at flower bud swelling stage with stem tip pruning is due to the fact that tip pruning reduces the auxin synthesis at the apex of branches, directing the transport of assimilates and cytokinins to the axillary buds of branches under flowering condition, inducing the formation of axillary inflorescences.

4. Gibberellic Acid (GA₃) (ng/g)

The results on endogenous Gibberellic acid concentration (ng/g) concentration after application of stem tip pruning and different bioregulators are presented in the Table 4 and Fig 4. The pooled data revealed significant difference among bioregulators with respect to gibberellic acid (GA₃) (ng/g) concentration at flower bud swelling stage of mango. Minimum (GA₃) (ng/g) concentration was recorded with application of paclobutrazol in combination with 6 benzyl amino purine (6-BA) (B₅) (1.08 ng/g) and paclobutrazol alone (1.08 ng/g). Maximum quantity of

(GA₃) (ng/g) was recorded with application of gibberellic acid (B₁) (1.48 ng/g). Similar reduction in GA₃ concentration with paclobutrazol was earlier reported by Upreti *et al.* (2013) [26] in mango cv. Totapuri, Srilatha and Reddy in mango cv. Raspuri and Vijaykrishna *et al.* (2020) [28] in mango cv. Banganpalli. Gibberellins, a group of plant growth hormones were reported to be inhibitory to flowering (Kachru) in mango and the available evidence suggests the flower promoting effect of paclobutrazol in mango is due to its anti-gibberellin activity. Triazole plant growth retardants like paclobutrazol are known for their ability to reduce growth by inhibiting gibberellin mediated cell elongation. Paclobutrazol inhibits gibberellin biosynthesis by blocking the oxidative conversion of kaurine to kaurenoic acid (Henry, 1985). Decrease in GA₃ content 6-BA might be due to altered isoprenoid pathway due to elevated levels of cytokinins at the flower bud swelling stage. Isoprenoid pathway associated with gibberellins biosynthesis also regulates partially the biosynthesis of other vital phytohormones such as abscisic acid (ABA) and cytokinins (Murti and Upreti, 2000) [26]. Floral induction is considered to be the result of elevated levels of up-regulated florigenic promoter (FP) and down-regulated vegetative promoter (VP), primarily gibberellins, whereas the reverse condition promotes vegetative growth (Nartvaranant *et al.*, 2000 and Davenport 2007) [12, 7]. Reduction in gibberellins content favouring floral bud formation has been reported previously by Abdel Rahim in mango, Sanjay in mango and Upreti *et al.* (2013) [26] in Totapuri mango. Decline in the gibberellin is an important contributor to floral bud initiation, and this is well substantiated by the studies of Tomer and Oosthuysen that gibberellic acid application reduced markedly the floral bud initiation and also prevented carbohydrate accumulation. Mishra and Dhillon found that an increase in the level of endogenous gibberellins will account for the production of solely male flowers, continuous vegetative growth and persistence of the malformed panicles on the tree.

Stem tip pruning has significantly influenced the gibberellic acid (GA₃) (ng/g) concentration at flower bud swelling stage of mango. Minimum gibberellic acid (GA₃) concentration was recorded in stem tip pruning (P₁) (1.20 ng/g) whereas, maximum gibberellic acid (GA₃) concentration was recorded in without stem tip pruning (P₁) (1.22 ng/g). Similar results of reduced endogenous gibberellic acid (GA₃) concentration with pruning was earlier reported by Sanjay *et al.* in mango cv. Amrapali, Srilatha and Reddy (2015) in mango cv. Raspuri. The removal of the apex of branches stimulates the budding of axillary buds and removes structures like gibberellins that inhibit reproductive buds. (Davenport, 2006) [6]

Significant differences were observed in the interaction effect between stem tip pruning and bioregulators with respect to (GA₃) (ng/g) content. Minimum (GA₃) (ng/g) concentration was recorded with application of paclobutrazol in combination with 6 benzyl amino purine (6-BA) (B₅) with stem tip pruning (P₁)

(1.07 ng/g) and paclobutrazol (B₃) with stem tip pruning (P₁) (1.07 ng/g). Maximum GA₃ (ng/g) concentration was recorded with application of gibberellic acid (B₁) without stem tip pruning (P₂) (1.53 ng/g). Lowest levels of GA₃ content in mango cv. Amrapali at flower bud swelling stage with moderate pruning was reported by Sanjay. The trees sprayed with Paclobutrazol and 6-BA, with the application of stem tip pruning utilized significantly resulted in increase in the per cent flowering over the control due to higher endogenous levels of ABA, Cytokinins (ZR and DHZR) and low levels of gibberellins (GA₃) during flower bud swelling (510) stage in the present investigation (Table 4.1.6). Hence, the results have clearly revealed that low levels of gibberellins (GA₃, higher endogenous levels of ABA, Cytokinins (ZR's and DHZR's) during flower bud swelling (510) stage is the condition required for flower induction in mango cv. Banganpalli.

5. Indole Acetic Acid (IAA) (ng/g)

The results on endogenous Indole Acetic Acid (IAA) (ng /g) concentration after application of stem tip pruning and different bioregulators are presented in the Table 5. The pooled data revealed that there was significant difference among bioregulators application with respect to Indole Acetic Acid (IAA) concentration at flower bud swelling stage. Among different bioregulators, maximum IAA concentration was recorded in the application of naphthalene acetic acid (B₄) (86.01 ng/g). Least quantity of IAA was recorded with control (B₈) (69.98 ng/g). Naphthalene acetic acid, being a synthetic auxin might have increased IAA content in the present investigation.

The pooled data has revealed significant difference in endogenous IAA concentration with respect to stem tip pruning. Maximum IAA concentration was recorded in without stem tip pruning (P₂) (80.03 ng/g) compared to with stem tip pruning (P₁) which has recorded minimum IAA concentration (P₁) (78.08 ng/g). Similar results were reported by Sanjay *et al.* (2009) who reported that the diffusible IAA decreased to a low level in shoot tips and ABA increased dramatically during early flower bud formation. Tip pruning reduces the auxin synthesis at the apex of branches, directing the transport of assimilates and cytokinins to the axillary buds of branches under flowering condition, inducing the formation of axillary inflorescences.

Significant differences were not observed in the interaction effect between stem tip pruning and bioregulators with respect to IAA content.

Hegele observed low IAA and GA concentrations parallel to elevated levels of active CKs in the buds might be the final link in the chain on the way to favourable hormonal conditions for floral induction in mango. CK conjugates, intermediately stored in wood and bark, might serve as a source to build up this high CKs to auxin+GAs ratio, which seems to be essential for successful floral induction.

Table 1: Effect of stem tip pruning and bio regulators on endogenous abscisic acid (ABA) (ng /g) concentration at flower bud swelling stage (510 stage of BBCH scale) of mango cv. Banganpalli

Abscicic acid (ABA) (ng /g)									
	2019			2020			Pooled		
Treatments	P ₁	P ₂	Mean of B	P ₁	P ₂	Mean of B	P ₁	P ₂	Mean of B
B ₁	65.13	62.70	63.92 ^g	69.47	67.60	68.53 ^f	67.30	65.15	66.23 ^h
B ₂	82.90	80.55	81.73 ^d	85.74	84.58	85.16 ^c	84.32	82.57	83.45 ^d
B ₃	91.13	87.97	89.55 ^b	90.13	87.65	88.89 ^b	90.63	87.81	89.22 ^b
B ₄	80.83	78.47	79.65 ^e	81.50	79.45	80.48 ^d	81.17	78.96	80.06 ^e
B ₅	92.73	90.80	91.77 ^a	90.94	89.48	90.21 ^a	91.84	90.14	90.99 ^a
B ₆	85.83	85.23	85.53 ^c	86.83	83.95	85.39 ^c	86.33	84.59	85.46 ^c
B ₇	74.50	71.53	73.02 ^f	76.85	74.05	75.45 ^e	75.68	72.79	74.23 ^g
B ₈	81.03	78.67	79.85 ^e	79.17	72.48	75.83 ^e	80.10	75.58	77.84 ^f
Mean of P	81.76 ^a	79.49 ^b		82.58 ^a	79.91 ^b		82.17 ^a	79.70 ^b	
Factors	S.Em ±	C.D. at 5%		S.Em ±	C.D. at 5%		S.Em ±	C.D. at 5%	
B	0.29	0.85		0.25	0.72		0.18	0.54	
P	0.15	0.42		0.12	0.36		0.09	0.27	
BXP	0.41	NS		0.35	1.01		0.26	0.76	

*Figures with same alphabet did not differ significantly

B ₁ : Gibberilic acid GA ₃ @ 50 ppm	B ₂ : 6 Benzylaminopurine BA @ 200 ppm	B ₃ : Paclobutrazol PBZ @ 1500 ppm
B ₄ : NaphthaleineaceticacidNAA @ 100 ppm	B ₅ : PBZ @ 1500 ppm+BA @ 200 ppm	B ₆ : PBZ @ 1500 ppm+ NAA @ 100 ppm
B ₇ : GA ₃ @ 50 ppm+ PBZ @ 1500 ppm	B ₈ : Control	
P ₁ : With stem tip pruning	P ₂ : without stem tip pruning	

Table 2: Effect of stem tip pruning and bio regulators on endogenous cytokinins (DHZR's) (Pg /g) concentration at flower bud swelling stage (510 stage of BBCH scale) of mango cv. Banganpalli

Dihydro Zeatin Ribose DHZR (Pg/g)									
	2019			2020			Pooled		
Treatments	P ₁	P ₂	Mean of B	P ₁	P ₂	Mean of B	P ₁	P ₂	Mean of B
B ₁	263.77	259.83	261.80 ^h	266.33	261.80	264.07 ^g	265.05	260.82	262.93 ^g
B ₂	300.22	298.63	299.43 ^c	303.00	300.30	301.65 ^b	301.61	299.47	300.54 ^b
B ₃	300.68	299.50	300.09 ^b	302.00	300.17	301.08 ^b	301.34	299.83	300.59 ^b
B ₄	296.27	293.83	295.05 ^e	298.27	295.83	297.05 ^d	297.27	294.83	296.05 ^d
B ₅	302.00	299.83	300.92 ^a	303.83	301.50	302.67 ^a	302.92	300.67	301.79 ^a
B ₆	297.03	295.50	296.27 ^d	298.83	296.83	297.83 ^c	297.93	296.17	297.05 ^c
B ₇	289.67	288.13	288.90 ^f	291.17	289.25	290.21 ^e	290.42	288.69	289.56 ^e
B ₈	267.63	264.97	266.30 ^g	268.50	267.00	267.75 ^f	268.07	265.98	267.03 ^f
Mean of B	289.66 ^a	287.53 ^b		291.49 ^a	289.09 ^b		290.58 ^a	288.31 ^b	
Factors	S.Em ±	C.D. at 5%		S.Em ±	C.D. at 5%		S.Em ±	C.D. at 5%	
B	0.25	0.73		0.19	0.55		0.18	0.53	
P	0.13	0.37		0.09	0.27		0.09	0.27	
BXP	0.36	1.04		0.27	0.77		0.26	0.75	

*Figures with same alphabet did not differ significantly

B ₁ : Gibberilic acid GA ₃ @ 50 ppm	B ₂ : 6 Benzylaminopurine BA @ 200 ppm	B ₃ : Paclobutrazol PBZ @ 1500 ppm
B ₄ : NaphthaleineaceticacidNAA @ 100 ppm	B ₅ : PBZ @ 1500 ppm+BA @ 200 ppm	B ₆ : PBZ @ 1500 ppm+ NAA @ 100 ppm
B ₇ : GA ₃ @ 50 ppm+ PBZ @ 1500 ppm	B ₈ : Control	
P ₁ : With stem tip pruning	P ₂ : without stem tip pruning	

Table 3: Effect of stem tip pruning and bio regulators on endogenous (ZR's) (Pg /g) concentration at flower bud swelling stage (510 stage of BBCH scale) of mango cv. Banganpalli

Zeatin Ribose (ZR Pg/g)									
	2019			2020			Pooled		
Treatments	P ₁	P ₂	Mean of B	P ₁	P ₂	Mean of B	P ₁	P ₂	Mean of B
B ₁	233.60	232.30	232.95 ^g	230.80	228.63	229.72 ^g	232.20	230.47	231.33 ^g
B ₂	259.97	257.50	258.73 ^e	257.90	254.50	256.20 ^e	258.93	256.00	257.47 ^e
B ₃	269.50	267.30	268.40 ^c	270.92	268.83	269.88 ^c	270.21	268.07	269.14 ^c
B ₄	263.83	262.63	263.23 ^d	263.83	261.47	262.65 ^d	263.83	262.05	262.94 ^d
B ₅	275.63	272.73	274.18 ^a	277.63	275.90	276.77 ^a	276.63	274.32	275.48 ^a
B ₆	270.75	269.42	270.08 ^b	272.57	270.42	271.49 ^b	271.66	269.92	270.79 ^b
B ₇	251.27	250.18	250.73 ^f	255.20	253.42	254.31 ^f	253.24	251.80	252.52 ^f
B ₈	236.80	228.97	232.88 ^g	232.82	226.00	229.41 ^g	234.81	227.48	231.15 ^g
Mean of P	257.67 ^a	255.13 ^b		257.71 ^a	254.90 ^b		257.69 ^a	255.01 ^b	
Factors	S.Em ±	C.D. at 5%		S.Em ±	C.D. at 5%		S.Em ±	C.D. at 5%	
B	0.26	0.76		0.24	0.68		0.19	0.56	
P	0.13	0.38		0.12	0.34		0.10	0.28	
BXP	0.37	1.07		0.33	0.96		0.27	0.79	

*Figures with same alphabet did not differ significantly

B ₁ : Gibberilic acid GA ₃ @ 50 ppm	B ₂ : 6 Benzylaminopurine BA @ 200 ppm	B ₃ : Paclobutrazol PBZ @ 1500 ppm
B ₄ : Naphthaleineacetic acid NAA @ 100 ppm	B ₅ : PBZ @ 1500 ppm+BA @ 200 ppm	B ₆ : PBZ @ 1500 ppm+ NAA @ 100 ppm
B ₇ : GA ₃ @ 50 ppm+ PBZ @ 1500 ppm	B ₈ : Control	
P ₁ : With stem tip pruning	P ₂ : without stem tip pruning	

Table 4: Effect of stem tip pruning and bio regulators on endogenous Gibberellic acid (GA₃) (ng/g) concentration at flower bud swelling stage (510 stage of BBCH scale) of mango cv. Banganpalli

Gibberellic acid GA ₃ (ng/g)									
	2019			2020			POOLED		
Treatments	P ₁	P ₂	Mean of B	P ₁	P ₂	Mean of B	P ₁	P ₂	Mean of B
B ₁	1.43	1.53	1.48 ^d	1.42	1.52	1.47 ^f	1.43	1.53	1.48 ^g
B ₂	1.15	1.15	1.15 ^b	1.17	1.18	1.18 ^c	1.16	1.17	1.17 ^d
B ₃	1.08	1.10	1.09 ^a	1.07	1.07	1.07 ^a	1.07	1.09	1.08 ^a
B ₄	1.14	1.14	1.14 ^b	1.15	1.16	1.16 ^b	1.15	1.15	1.15 ^c
B ₅	1.07	1.09	1.08 ^a	1.06	1.07	1.07 ^a	1.07	1.08	1.08 ^a
B ₆	1.12	1.13	1.12 ^b	1.13	1.14	1.14 ^b	1.13	1.13	1.13 ^b
B ₇	1.31	1.33	1.32 ^c	1.27	1.21	1.24 ^d	1.30	1.27	1.28 ^e
B ₈	1.28	1.31	1.30 ^c	1.30	1.37	1.33 ^e	1.30	1.34	1.32 ^f
Mean of P	1.20 ^a	1.22 ^b		1.20 ^a	1.21 ^a		1.20 ^a	1.22 ^b	
Factors	S.Em ±	C.D. at 5%		S.Em ±	C.D. at 5%		S.Em ±	C.D. at 5%	
B	0.01	0.02		0.01	0.02		0.00	0.01	
P	0.00	0.01		0.00	0.01		0.00	0.01	
BXP	0.01	0.02		0.01	0.03		0.01	0.02	

*Figures with same alphabet did not differ significantly

B ₁ : Gibberilic acid GA ₃ @ 50 ppm	B ₂ : 6 Benzylaminopurine BA @ 200 ppm	B ₃ : Paclobutrazol PBZ @ 1500 ppm
B ₄ : Naphthaleineacetic acid NAA @ 100 ppm	B ₅ : PBZ @ 1500 ppm+BA @ 200 ppm	B ₆ : PBZ @ 1500 ppm+ NAA @ 100 ppm
B ₇ : GA ₃ @ 50 ppm+ PBZ @ 1500 ppm	B ₈ : Control	
P ₁ : With stem tip pruning	P ₂ : without stem tip pruning	

Table 5: Effect of stem tip pruning and bio regulators on endogenous auxin (IAA) (ng /g) concentration at flower bud swelling stage (510 stage of BBCH scale) of mango cv. Banganpalli.

Indole acetic acid (IAA) ng/g									
	2019			2020			POOLED		
Treatments	P ₁	P ₂	Mean of B	P ₁	P ₂	Mean of B	P ₁	P ₂	Mean of B
B ₁	68.80	70.50	69.65 ^g	73.26	74.33	73.79 ^e	71.03	72.42	71.72 ^f
B ₂	79.83	81.50	80.67 ^e	79.34	82.63	80.98 ^d	79.59	82.07	80.83 ^d
B ₃	82.37	84.28	83.33 ^c	81.89	83.93	82.91 ^c	82.13	84.11	83.12 ^b
B ₄	85.65	87.16	86.41 ^a	84.82	86.42	85.62 ^a	85.23	86.79	86.01 ^a
B ₅	80.03	82.58	81.31 ^d	82.6	84.71	83.65 ^b	81.32	83.65	82.48 ^c
B ₆	83.6	86.14	84.87 ^b	80.33	81.49	80.91 ^d	81.97	83.82	82.89 ^b
B ₇	73.46	75.52	74.49 ^f	75.85	76.75	76.30 ^e	74.65	76.13	75.39 ^e
B ₈	67.75	71.71	69.73 ^g	69.62	70.83	70.22 ^f	68.69	71.27	69.98 ^g
Mean of P	77.69 ^b	79.92 ^a		78.46 ^b	80.13 ^a		78.08 ^b	80.03 ^a	
Factors	S.Em ±	C.D. at 5%		S.Em ±	C.D. at 5%		S.Em ±	C.D. at 5%	
B	0.20	0.58		0.22	0.64		0.16	0.47	
P	0.10	0.29		0.11	0.32		0.08	0.24	
BXP	0.28	0.82		0.31	0.91		0.23	NS	

*Figures with same alphabet did not differ significantly

B ₁ : Gibberilic acid GA ₃ @ 50 ppm	B ₂ : 6 Benzylaminopurine BA @ 200 ppm	B ₃ : Paclobutrazol PBZ @ 1500 ppm
B ₄ : Naphthaleineacetic acid NAA @ 100 ppm	B ₅ : PBZ @ 1500 ppm+BA @ 200 ppm	B ₆ : PBZ @ 1500 ppm+ NAA @ 100 ppm
B ₇ : GA ₃ @ 50 ppm+ PBZ @ 1500 ppm	B ₈ : Control	
P ₁ : With stem tip pruning	P ₂ : without stem tip pruning	

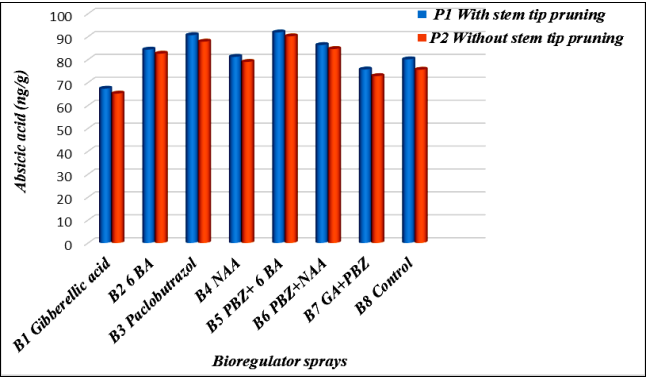


Fig 1: Effect of stem tip pruning and bioregulators on ABA concentration (ng /g) at flower bud swelling stage (510 stage of BBCH scale) of mango cv. Banganpalli.

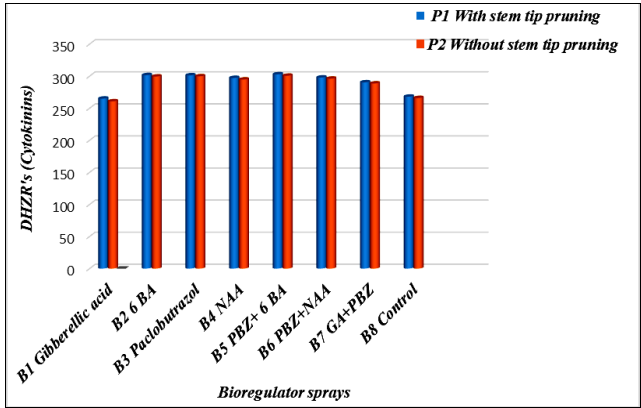


Fig 2: Effect of stem tip pruning and bioregulators on DHZR concentration (Pg /g) at flower bud swelling stage (510 stage of BBCH scale) of mango cv. Banganpalli.

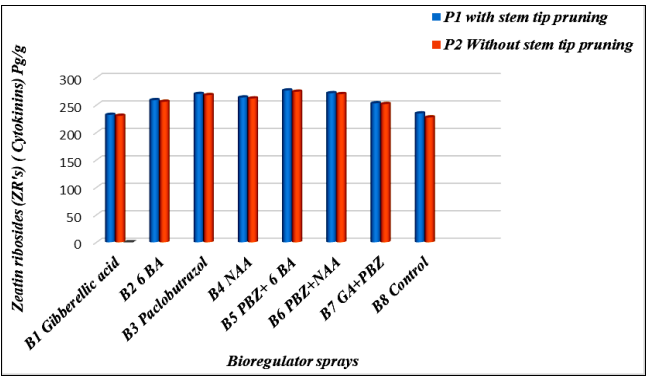


Fig 3: Effect of stem tip pruning and bioregulators on ZR concentration (Pg /g) at flower bud swelling stage (510 stage of BBCH scale) of mango cv. Banganpalli.

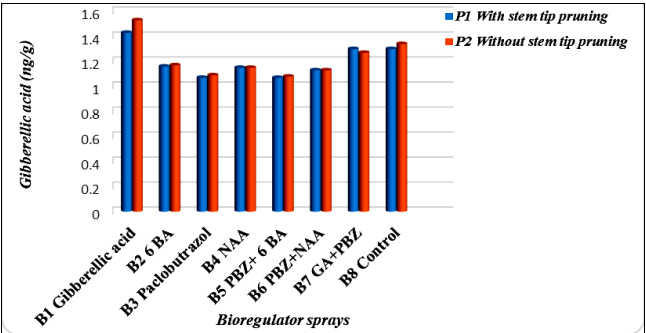


Fig 4: Effect of stem tip pruning and bioregulators on gibberellic acid GA₃ (ng/g) concentration at flower bud swelling stage (510 stage of BBCH scale) of mango cv. Banganpalli.



Plate 1: Foliar application of Bioregulators



Plate 2: Stem tip pruning at 5 cm level

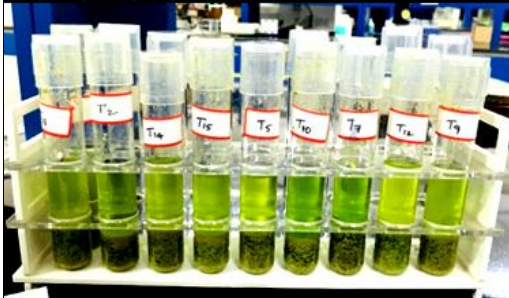
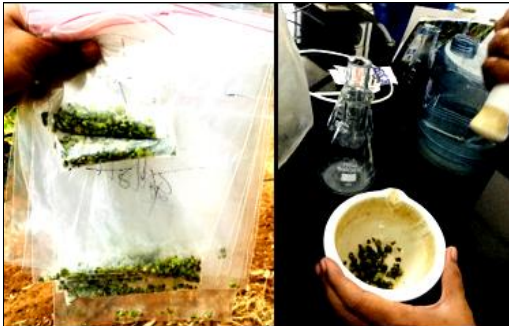


Plate 3: View of Collection of swollen buds and preparation of samples for hormonal analysis at flower bud swelling stage (510 stage of BBCH scale).

Conclusion

The present study indicated that application of paclobutrazol in combination with 6 benzyl aminopurine (6-BA) (B₅) along with stem tip pruning (P₁) during flower bud differentiation stage has significantly resulted in highest endogenous ABA, cytokinins, lowest GA₃ contents at flower bud swelling stage (510 stage of BBCH scale).

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