Pruning and Paclobutrazol Induced Flowering and Changes in Phenols and Flavonoids of Mango (Mangifera indica L.) cv. Raspuri

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Abstract
Pruning and use of paclobutrazol (PBZ) have been identified as common strategies for enhanced flowering and productivity enhancement in mango. The present study was aimed to investigate the effects of pruning (current and previous season’s vegetative growth) and PBZ (3 ml/m canopy diameter) on flowering, yield and leaf phenol contents in mango cv. Raspuri. Early flowering as a result of PBZ application led to advanced fruit harvest by 20.4 days in trees pruned to current season’s growth followed by 20.7 days in unpruned trees. Drastic increase in total phenolics, total flavonoids and phenolic acid contents were observed at 75 days after PBZ application in PBZ treated unpruned trees and in trees pruned to current season’s growth. The high levels of coumaric acid, 4-hydroxy benzoic acid and salysilic acid and low levels of caffeic acid and t-cinnamic acid were observed following PBZ application in tree pruned to current season’s growth and unpruned trees compared to control. Studies indicated that, the pruning of trees to current season’s growth and PBZ application can influence the phenols and flavonoids contents of mango.

Keywords: mango, pruning, paclobutrazol, flowering, phenolic acids, flavonoids.

Introduction
Mango (Mangifera indica L.) is commercially important fruit crop of India cultivated in area of 2.50 million ha with a production of 18.08 million tonnes (Anon, 2013). Excessive vegetative growth causes less flowering as a result of unmanageable and large tree size. Pruning and the growth retardants are the two simple and effective means for enhancing flowering. From the available literature is was evident that, most of the reports in mango targeted pruning application as a means of induction in early and regular flowering (Singh et al., 2009; Balamohan and Gopu, 2014) and pruning effects on biochemical parameters have not been reported. The paclobutrazol induced flowering responses have been reported as the consequences of modifications in physiological activities as well as changes in cellular metabolites (Nafeez et al., 2010; Abdel Rahim et al., 2011; Upreti et al., 2013). However, the possible mechanism by which phenols exerts such effects are less understood.

In the present investigation, attempts have been made to investigate effects of pruning and paclobutrazol alone and in combination on flowering, yield, phenols and flavonoids contents in mango cv. Raspuri with a view to understand role of phenols and flavanoids in flowering manipulations of mango.

Material And Methods
The investigations were conducted at the experimental farm of Indian Institute of Horticultural Research, Bangalore on 4 years old trees of mango cv. Raspuri grafted on Olour rootstock and spaced at 7 X 7 m distance during the years 2013 - 2014. The experiment was laid out with three replications and six treatments in a factorial randomized block design with various combinations of pruning (pruning to current season’s growth, pruning of previous season’s growth and no pruning) and paclobutrazol (PBZ) application at @ 3 ml/m canopy diameter. Six trees were maintained under each treatment. Pruning was carried out during 3rd week of July, 2013. PBZ (25% w/v a.i.; Zeneca Limited, Surry, UK) was applied once as soil drench during the month of September, 2013 by spreading in a circular band of 25 cm width at a radial distance of 75 cm from the tree trunk. Only water was applied to the control plants. The different treatment combinations used were T1- pruning to current season’s growth + PBZ application @ 3 ml/m canopy diameter, T2- pruning to current season’s growth, T3- pruning to previous season’s growth + PBZ application @ 3 ml/m canopy diameter, T4- pruning of previous season’s growth, T5- no pruning + PBZ application @ 3 ml/m canopy diameter, T6- no pruning and no PBZ application (control). During the experimentation, the average maximum and minimum temperatures were 29.4 and 19 ºC respectively, relative humidity 74.5 % and total rainfall 732.7 mm. After the emergence of new shoots, 50 shoots were tagged in all directions and observations on days for 50% flowering and percent flowering were recorded from tagged shoots. Data on number of days from flowering to harvest and yield were also recorded. Besides, leaf samples at 75 days after paclobutrazol application were drawn for determining the contents of total phenols, total flavonoids and phenolic acids.

Estimation Of Total Phenols
Total phenols were estimated spectrophotometrically using Folin-Ciocalteu reagent according to Bray and
Temperature. The extract was centrifuged at 5000 rpm at room temperature and the residue was re-extracted again with another 50 ml of 80% methanol. The supernatants were pooled and dried in a rotary evaporator at 40 °C under reduced pressure. The residue was dissolved in 30 ml distill water and pH adjusted to 2.8 with 6 M HCl.

The free phenolic acids were extracted 3 times with 30 ml of chilled diethyl ether. The ethereal portion was collected and further evaporated to dryness under vacuum at 40 °C. The residue was dissolved in 2 ml HPLC grade methanol and filtered through 0.2 µm syringe filter (Millipore, USA) for HPLC analysis. The HPLC system (Prominence, Shimadzu, Japan) employed had photodiode array detector (SPD-M20A) and reverse phase C18 column (5 µm, 4.6 X 250 mm, Supelco, USA). Both the detector and column temperatures were set at 40 °C. The detector was operated at 280 nm. A binary gradient of solvent system consisted mobile phase A with 10% methanol and B methanol/acetic acid (97.5: 2.5 v/v). The gradient was isocratic with A for 10 min followed linear increase of B in A to 18% for 15 min, 70% for 20 min, 75% for 30 min, 80% for 35 min and 100% B for 40 min at the uniform flow rate of 1.0 ml/min. The 10 phenolic acids namely o-caumaric acid, gallic acid, pyrocatechol, 4-hydroxy benzoic acid, caffeic acid, salicylic acid, rosmarinic acid, t-ferrulic acid, sinapic acid and cinnamic acid were quantified by comparing their respective retention times and peak areas using their standards (Sigma-Aldrich, USA).

Results And Discussion

Flowering characters: Effects of pruning and paclobutrazol were significant with respect to % flowering shoots and number of days for 50% flowering (Table 1). However their interaction effects were non-significant. T1 and T3 treatments were at par with respect to% flowering shoots and number of days for 50% flowering and flowering percentage. T1 advanced the number of days for 50% flowering by 17 days followed by T5 with 14.3 days. Among the pruning levels, removal of current season’s growth recorded early flowering than the trees pruned to previous season’s growth. More number of days taken for 50% flowering in severly pruned trees might be because of greater utilization of available carbohydrates for vegetative growth at the expense of flowering and longer time taken to replenish the carbohydrates lost in pruning. Our results are in accordance with Balamohan and Gopu (2014) and Jannoyer (2009), who reported that, severe pruning delayed the flowering in mango. Early and intense flowering induced by PBZ may be the consequence of early shoot maturity and increased photosynthesis rate (Singh & Singh 2009), carbohydrate accumulation (Abdel Rahim et al., 2011) and decline in flowering reducing hormone, gibberellins (Upreti, et al., 2013).

Number of days from flowering to harvest ranged between 128.3-149.3 days under the different treatments (Table 1). Pruning did not show much influence on number of days from flowering to harvest, however PBZ significantly reduced the number of days from flowering to harvesting. However, the interaction effects of pruning and PBZ were non-significant. The PBZ induced early flowering has been reported by Upreti et al. (2013) in Totapuri. The early flowering in T1 advanced the harvesting of fruits by 20.4 days followed by 20.7 days in T5 when compared with control (T6).

Yield attributes: The paclobutrazol effects and interaction of pruning and PBZ were found significant with respect to fruit number per tree and yield per tree (Table 1). The treatment T3 recorded 66.8% higher fruit yield followed by T1 (26.7%). The higher yields in the PBZ treated trees is ascribed due to high flowering intensity which resulted higher fruit number. Yield advantage following paclobutrazol application was in agreement with the findings of Upreti et al. (2013) and Sarkar and Rahim (2012) in different mango varieties.

Total Phenols and flavonoids: Effects of pruning, paclobutrazol and their interaction were significant on total phenol content (Table 1). Unpruned trees with PBZ application (T3) recorded 32.66% higher phenol content followed by trees pruned to current season’s growth with PBZ application (T1) compared to control (T6). The induction in phenol content by PBZ concomittant with early and profuse flowering revealed the possible involvement phenols in mango flowering. Higher phenols during flowering has been reported by Palanichamy et al.(2012) and Patil et al. (1992) in different mango cultivars. The PBZ induced increase in phenols may not beof direct effect of PBZ on phenol biosynthesis but rather through its effects on phytohormone mediated increase in phenol content as stated by Rademacher (2000). The increased phenol contents are inhibitory to growth through its negative effects on cell division and cell elongation processes. However, it needs to be clarified how increased phenols are directly associated with increased flowering.

Estimation Of Total Flavonoids (TF)

TF content was estimated spectrophotometrically as per Zhishen et al. (1999) and the values were expressed as mg/g fresh weight.

Estimation Of Phenolic Acids

Estimation of different phenolic acids was carried out using High Pressure Liquid Chromatography. Leaf samples were extracted according to Weidner et al. (1999). Fresh leaf sample (5 g) was homogenized in liquid nitrogen and the phenolic acids were extracted in 50 ml of 80% chilled methanol (v/v) for one hour at room temperature. The extract was centrifuged at 5000 rpm at room temperature and the residue was re-extracted again with another 50 ml of 80% methanol. The supernatants were pooled and dried in a rotary evaporator at 40 °C.
Significant differences in flavonoid content due to PBZ was recorded, but the interaction effect was non-significant (Table 1). The highest flavonoid content to the tune of 9.42 mg/g was noticed in unpruned trees with PBZ (T5) followed by trees pruned to current season’s growth (T1). Flavonoid content increased drastically in all the PBZ treated trees than the control trees. The PBZ induced increase in flavonoid content showed that the PBZ induced changes in growth determining characters and flowering is mediated through accumulations in flavonoids. However it needs to be ascertained how such accumulation in flavonoids triggers such responses.

Phenolic Acids
The composition and quantity of phenolic acids in leaves greatly varied in control and treatments (Table 2). The prominent phenolic acids determined were 4-hydroxy benzoic acid, o-caumaric acid, salicylic acid, caffeic acid and t-cinnamic acid. PBZ was more responsive in altering the composition of phenolic acids as compared to pruning. Under T5 treatment 4-hydroxy benzoic acid (79.84 µg/g), coumaric acid (65.21 µg/g) and salicylic acid (6.75 µg/g) contents were increased, but the contents of caffeic acid (1.39 µg/g) and cinnamic acid (0.41 µg/g) were decreased compared to control. From the results it was evident that higher leves of 4-hydroxy benzoic acid, coumaric acid and salicylic acid content contributed to the increased levels of total phenols under PBZ treatment. Such changes in phenolic acids may be the result of sensitization of phenolic acid biosynthetic pathway by paclobutrazol treatment. The results also depicted that 4-hydroxy benzoic acid, coumaric acid and salicylic acid were some of the phenolic acids contributed to PBZ induced flowering in mango. The above results are in line with the findings of Mert et al. (2013) that hydroxycinnamnic acid and p-caumaric acid contents are high in olive trees during ‘on’ year as compared to ‘off’ year.

Conclusion
From the study, it was concluded that the pruning of current season’s growth and PBZ application are vital for induction of early flowering and advancing fruit harvest in mango and such beneficial effects of treatments mediated through increases in phenolics and flavonoids contents.

References
[13] S.R. Weidner, R. Amarowicz, M. Karamac and G. Dabrowski, Phenolic acids in caryopses of two cul-


Table 1. Effects of pruning and paclobutrazol on flowering characters in mango cv Raspuri

<table>
<thead>
<tr>
<th>Treatments</th>
<th>% flowering shoots</th>
<th>Days to 50% flowering</th>
<th>No. of days from flowering to harvest</th>
<th>Number of fruits/plant</th>
<th>Yield/plant (kg)</th>
<th>Total Phenolic content (mg g⁻¹)</th>
<th>Total flavonoid content (mg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>82.8</td>
<td>135.6</td>
<td>128.3</td>
<td>103.66</td>
<td>21.33</td>
<td>48.43</td>
<td>9.71</td>
</tr>
<tr>
<td>T₂</td>
<td>42.2</td>
<td>159.3</td>
<td>148.3</td>
<td>29.33</td>
<td>5.4</td>
<td>39.97</td>
<td>7.69</td>
</tr>
<tr>
<td>T₃</td>
<td>65.2</td>
<td>145.6</td>
<td>130.3</td>
<td>78.0</td>
<td>15.66</td>
<td>45.13</td>
<td>8.30</td>
</tr>
<tr>
<td>T₄</td>
<td>23.9</td>
<td>160.6</td>
<td>149.3</td>
<td>9.33</td>
<td>1.86</td>
<td>33.73</td>
<td>6.86</td>
</tr>
<tr>
<td>T₅</td>
<td>79.9</td>
<td>138.3</td>
<td>128.6</td>
<td>146.66</td>
<td>28.08</td>
<td>50.57</td>
<td>9.42</td>
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<tr>
<td>T₆</td>
<td>49.4</td>
<td>152.6</td>
<td>149.0</td>
<td>57.5</td>
<td>16.83</td>
<td>38.12</td>
<td>7.41</td>
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<td>Sem+</td>
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<tr>
<td>Pruning</td>
<td>3.68</td>
<td>1.03</td>
<td>0.71</td>
<td>16.96</td>
<td>3.33</td>
<td>6.27</td>
<td>1.43</td>
</tr>
<tr>
<td>PBZ</td>
<td>3.01</td>
<td>0.84</td>
<td>0.58</td>
<td>13.85</td>
<td>2.72</td>
<td>5.12</td>
<td>1.17</td>
</tr>
<tr>
<td>Pruning X</td>
<td>5.21</td>
<td>1.4</td>
<td>1.01</td>
<td>23.98</td>
<td>4.71</td>
<td>8.87</td>
<td>2.03</td>
</tr>
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<td>PBZ</td>
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<tr>
<td>Pruning</td>
<td>11.6</td>
<td>3.26</td>
<td>2.26</td>
<td>NS</td>
<td>NS</td>
<td>0.19</td>
<td>NS</td>
</tr>
<tr>
<td>PBZ</td>
<td>9.48</td>
<td>2.66</td>
<td>1.84</td>
<td>62.06</td>
<td>8.58</td>
<td>0.16</td>
<td>3.69</td>
</tr>
<tr>
<td>Pruning X</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>107.49</td>
<td>NS</td>
<td>0.27</td>
<td>NS</td>
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<tr>
<td>PBZ</td>
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<td></td>
<td></td>
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</tbody>
</table>

*PBZ- paclobutrazol
T₁ – pruning of current season’s growth + soil application of PBZ @ 3 ml/m canopy diameter
T₂ – pruning of current season’s growth
T₃ – pruning of previous season’s growth + soil application of PBZ @ 3 ml/m canopy diameter
T₄ – pruning of previous season’s growth
T₅ – no pruning + soil application of PBZ @ 3 ml/m canopy diameter
T₆ – no pruning + no PBZ (control)
Table 1. Effects of pruning and paclobutrazol on phenolic acid levels in mango cv. Raspuri (µg g\(^{-1}\)) (data represents mean ± SE)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Gallic acid</th>
<th>4-hydroxy BA</th>
<th>Caffeic acid</th>
<th>Salicylic acid</th>
<th>t-ferrulic acid</th>
<th>Sinapic acid</th>
<th>Rosmarinic acid</th>
<th>o-caumaaric acid</th>
<th>t-cinnamic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>T(_1)</td>
<td>ND</td>
<td>64.32±0.16</td>
<td>1.39±0.29</td>
<td>ND</td>
<td>0.97±0.04</td>
<td>ND</td>
<td>4.78±0.10</td>
<td>ND</td>
<td>47.79±0.11</td>
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<td>T(_2)</td>
<td>31.34±0.26</td>
<td>ND</td>
<td>ND</td>
<td>0.72±0.04</td>
<td>3.72±0.76</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>39.8±0.11</td>
</tr>
<tr>
<td>T(_3)</td>
<td>ND</td>
<td>19.91±0.16</td>
<td>ND</td>
<td>0.64±0.04</td>
<td>ND</td>
<td>44.09±0.10</td>
<td>4.27±0.78</td>
<td>ND</td>
<td>11.45±0.11</td>
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<tr>
<td>T(_4)</td>
<td>ND</td>
<td>9.42±0.29</td>
<td>7.50±0.04</td>
<td>ND</td>
<td>0.85±0.10</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.86±0.11</td>
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<tr>
<td>T(_5)</td>
<td>ND</td>
<td>79.84±0.16</td>
<td>0.85±0.29</td>
<td>6.75±0.04</td>
<td>ND</td>
<td>3.57±0.10</td>
<td>5.18±0.78</td>
<td>ND</td>
<td>63.21±0.11</td>
</tr>
<tr>
<td>T(_6)</td>
<td>ND</td>
<td>4.07±0.16</td>
<td>15.48±0.29</td>
<td>1.97±0.04</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.63±0.11</td>
</tr>
</tbody>
</table>

ND- not detected

T\(_1\) – pruning of current season’s growth + soil application of PBZ @ 3 ml/m canopy diameter
T\(_2\) – pruning of current season’s growth
T\(_3\) – pruning of previous season’s growth + soil application of PBZ @ 3 ml/m canopy diameter
T\(_4\) – pruning of previous season’s growth
T\(_5\) – no pruning + soil application of PBZ @ 3 ml/m canopy diameter
T\(_6\) – no pruning + no PBZ (control)