ESTIMATION OF MDA, TOTAL CHLOROPHYLL AND METHIONINE CONTENT IN RELATION TO MALFORMATION DISORDER OF MANGO (Mangifera indica L.) UNDER LOW TEMPERATURE AND HIGH RELATIVE HUMIDITY CONDITION PREVAILING IN DIFFERENT STATES OF NORTH INDIA

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Abstract - Malformation disorder of mango occurs as a result of surge of ethylene synthesis in plants following biotic and abiotic stresses like pathogen attack, chilling, high relative humidity etc. The increased level of ethylene brings about physiological alteration in plants. In malformed tissue samples of five commercial mango varieties collected from different states of north India recorded an increase in MDA (malondialdehyde) and methionine while a reduced content of chlorophyll.

Keywords - Mango malformation, MDA, methionine, total chlorophyll, stress ethylene, low temperature, high R.H

I. INTRODUCTION

Mango (Mangifera indica L.) is an important fruit crop of tropical and subtropical areas of the world. It is extensively cultivated in many mango growing provinces, but due to a deadly disorder of mango known as malformation, its productivity has dropped to a meager value, causing heavy economic losses on global basis (NHB, Indian Horticulture Database, 2011). The malady appears in two forms viz, vegetative and floral. In vegetative malformation there is multi branching of shoot apex with scaly leaves leading to Bunchy Top or Witch’s broom (Bhatnagar and Beniwal, 1977). Floral malformation shares the same symptom of multi branching of rachis and bears mostly male flowers and rarely bisexual. In malformed panicles flowers are sterile and thus bear mostly male flowers and rarely bisexual flowers in floral malformation. In vegetative malformation due to several biotic and abiotic stresses is the main causal agent of malformation. As a result of stress condition, several biochemical compounds accumulate in plants. The present study was undertaken to estimate the amount of MDA, total chlorophyll and methionine in abiotic stressed plants in five states of northern India.

II. MATERIAL AND METHODS

The study of weather parameters was conducted in five states of India namely Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi. For these location climatic statistics pertaining to maximal and minimal temperature, RH and Wind velocity, have been gathered for a year i.e. 2016, for the months of February and March (during flower initiation to flowering period). Five varieties of mango namely Amrapali, Dasher, Langra, Chausa and Bombay Green were selected. The experimental material used was healthy and malformed leaf tissues and was collected from Mango orchards of Pantnagar, Uttarakhand; Ranchi, Jharkhand; New Delhi, Allahabad, Uttar Pradesh and Patna, Bihar.

Malondialdehyde (MDA) Content

Procedure

The amount of MDA produced by thiobarbituric acid reaction was used to determine lipid peroxidation, by method given by Heath and packer (1968). 0.2 gm of fresh sample was homogenized in 3mL 0.1% TCA. The centrifugation of homogenized mixture was done at 10,000 rpm for 10 min. 1.2mL of (0.5% TBA in twenty percent TCA) was added to 0.3mL supernatant and it was then incubated in water bath for 30 min at 95°C. Ice was used to terminate the reaction. Absorbance was read at 532 and 600 nm. The concentration of MDA was estimated using the extinction coefficient of 155mM^-1cm^-1, after subtracting the non specific absorbance at 600 nm.

Chlorophyll Content

Procedure

Chlorophyll content was determined in leaves by a method described by Hiscox et al. (1979). For this 50 mg of finely chopped leaf tissues were taken in test tubes. 10 mL of dimethyl sulfoxide was added to each test tubes and it was incubated at 65°C for three hours in an oven. Incubation of three hours was then followed by determining the absorbance of DMSO
containing chlorophyll at 663 nm and 645 nm using a spectrophotometer. Pure DMSO was used as blank.

**Methionine Content Procedure**

Methionine content was determined by method prescribed by Horn *et al.* (1946), with some modifications. 0.5 gms defatted samples were weighed in fifty millilitre conical flasks. Six millilitre of 2N hydrogen chloride was added and was autoclaved at 15lb pressure for 1 hr and small amount of activated charcoal was mixed and the hydrolysate was boiled and washed with warm water. The filtrate was neutralized with 10N sodium hydroxide to pH 6.5. The volume was made to 50 ml with water after lowering to air temperature and 25 ml of it was transferred to 100ml conical flask. 3ml of ten percent sodium hydroxide was added to it followed by 0.15ml sodium nitroprusside (10%). After 10 min 1ml of glycine (3%) solution was added. After another 10 min 2ml of orthophosphoric acid was added and shaken vigorously. Intensity of red color after 10 min was read at 520 nm against a blank prepared in the same way without nitroprusside.

**Statistical analysis**

The data were analyzed statistically by two factor Randomized Block Design (RBD).

**III. RESULTS**

The study of weather parameters in five states of northern India revealed that in the entire region during flower initiation to flowering period (February- March) the weather variables were highly favorable for malformation disorder of mango. Low temperature (maximum and minimum), high relative humidity and feeble wind favored the occurrence of malformation (Table 1). Similarly in the healthy vegetative tissue of cultivar, Dasheri, it was found to be 62.23, 61.24, 55.75, 53.25 and 55.25 mg phenol/ 100 g of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi respectively and that in malformed vegetative tissue was 82.25, 75.25, 78.15, 78.25 and 66.28 mg phenol/ 100 g of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively. In the healthy vegetative tissue of Langra it was 53.25, 70.16, 56.93, 60.75 and 56.75 mg phenol/ 100 g of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively, and that in malformed vegetative tissue was 67.60, 69.10, 95.28, 94.68 and 92.47 mg phenol/ 100 g of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively.

**Malondialdehyde content (µmol g⁻¹ fresh weight) in malformed and healthy leaf tissue samples**

Malondialdehyde content in healthy and malformed tissues was estimated over two months (February-March) during flower initiation to flowering period in Amrapali, Dasheri, Langra, Chausa and Bombay Green cultivars of mango in different states (Table 1 and Fig 1).

As may be observed, the Malondialdehyde content in malformed tissue was higher as compared to healthy tissue in all the cultivars in different states. Malondialdehyde content in healthy vegetative tissue of Amrapali was 0.114, 0.116, 0.115, 0.121 and 0.117 µmol g⁻¹ fresh weight of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi respectively and that in malformed vegetative tissue was 0.436, 0.475, 0.425, 0.417, 0.435 µmol g⁻¹ fresh weight of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively.

Similarly in the healthy vegetative tissue of cultivar, Dasheri, it was found to be 3.17, 3.32, 3.24, 3.15 and 3.12 mg phenol/ 100 g of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively. Similarly, in the healthy vegetative tissue of cultivar, Chausa it was found to be 71.80, 52.25, 61.72, 61.82 and 58.73 mg phenol/ 100 g of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi respectively and that in malformed vegetative tissue was 82.25, 75.25, 78.15, 78.25 and 66.28 mg phenol/ 100 g of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively. In the healthy vegetative tissue of Bombay Green it was 57.00, 58.60, 70.96, 72.96 and 61.85mg phenol/ 100 g of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi respectively, and that in malformed vegetative tissue was 76.00, 78.00, 94.10, 69.45 and 76.02 mg phenol/ 100 g of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi respectively.

**Total chlorophyll content (mg g⁻¹ fw) in malformed and healthy leaf tissue samples**

The total chlorophyll content in healthy and malformed tissues was estimated over two months (February-March) during flower initiation to flowering period in Amrapali, Dasheri, Langra, Chausa and Bombay Green cultivars of mango in different states (Table 2 and Fig 2). As may be observed, the Total chlorophyll content in malformed tissue was lower as compared to healthy tissue in all the cultivars in different states. Total chlorophyll content in healthy vegetative tissue of Amrapali was 3.15, 3.15, 3.12, 3.12 and 3.12 mg g⁻¹ fw of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi respectively and that in malformed vegetative tissue was 2.34, 2.35, 2.51, 2.48 and 2.35 mg g⁻¹ fw of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi respectively.

Similarly in the healthy vegetative tissue of cultivar, Dasheri, it was found to be 3.17, 3.32, 3.24, 3.15 and 3.12 mg g⁻¹fw of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi respectively.
3.15 mg g⁻¹ fw of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi respectively and that in malformed vegetative tissue was 2.38, 2.74, 2.63, 2.51 and 2.45 mg g⁻¹ fw of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi respectively. In the healthy vegetative tissue of Langra it was 3.20, 3.11, 3.11, 3.18 and 3.17 mg g⁻¹ fw of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi respectively, and that in malformed vegetative tissue was 2.97, 2.15, 2.72, 2.75 and 2.57 mg g⁻¹ fw of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi respectively.

Similarly, in the healthy vegetative tissue of cultivar, Chausa it was found to be 3.30, 3.35, 3.443.15 and 3.13 mg g⁻¹ fw of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively, and that in malformed vegetative tissue was 2.48, 2.60, 2.80, 2.45 and 2.40 mg g⁻¹ fw of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi respectively. In the healthy vegetative tissue of Bombay Green it was 3.18, 3.11, 3.12, 3.17 and 3.16 mg g⁻¹ fw of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi respectively, and that in malformed vegetative tissue was 2.53, 2.25, 2.56, 2.78 and 2.45 mg g⁻¹ fw of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi respectively.

**Methionine content (µg g⁻¹ fresh weight) in malformed and healthy leaf tissue samples**

The Methionine content in healthy and malformed tissues was estimated over two months (February-March) during flowering initiation to flowering period in Amrapali, Dasheri, Langra, Chausa and Bombay Green cultivars of mango in different states (Table 3 and Fig 3).

As may be observed, the Methionine content in malformed tissue was higher as compared to healthy tissue in all the cultivars in different states. Methionine content in healthy vegetative tissue of Amrapali was 62.65, 62.11, 63.14, 62.15 and 62.15 µg g⁻¹ fw of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi respectively and that in malformed vegetative tissue was 94.35, 95.75, 96.00, 95.00 and 95.62 µg g⁻¹ fw of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi respectively.

Similarly in the healthy vegetative tissue of cultivar, Dasheri, it was found to be 65.35, 63.72, 62.81, 62.82 and 60.70 µg g⁻¹ fw of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi respectively and that in malformed vegetative tissue was 2.38, 2.74, 2.63, 2.51 and 2.45 mg g⁻¹ fw of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi respectively.

In the healthy vegetative tissue of Langra it was 64.72, 64.35, 66.34, 63.00 and 62.15 µg g⁻¹ fw of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively, and that in malformed vegetative tissue was 100.72, 99.95, 100.75, 96.00 and 100.02 µg g⁻¹ fw of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively.

In the healthy vegetative tissue of Chausa it was 63.89, 63.61, 65.42, 65.22 and 63.15 µg g⁻¹ fw of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi respectively, and that in malformed vegetative tissue was 98.72, 100.21, 99.45, 100.25 and 99.65 µg g⁻¹ fw of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi respectively.

**IV. DISCUSSION**

In an another finding, higher content of malondialdehyde was recorded in maize genotypes under water stress condition as compared to control ones (Helal Ragab Moussa and Samir Moustafa abdel-Aziz, 2008). Increase in MDA content under stress condition advocates that stress causes harm to membrane, due to lipid peroxidation by means of ROS (Sairam et al., 2000). Drastic increase in free radicals leads to leaky membrane which causes lipid peroxidation (Smirnoff, 1993). Result of a finding suggested that salinity stress affects lipid peroxidation in leaves and roots of groundnut (Jain et al., 2001).

Stability of chlorophyll depends on the stability of membrane. Being a membrane bound pigment, chlorophyll is lost due to breakdown of membrane under stress condition. Under salinity stress condition, reduction in chlorophyll content was observed (Molazem et al., 2010). In an another finding it was reported that there is decrease in chlorophyll content under stress condition (Ommen et al., 1999). In an another study, large decline in the content of chlorophyll a, chlorophyll b and total chlorophyll was reported under water stress condition in Sunflower (Manivannan et al., 2007). Similar result was observed in genotypes of bread wheat under lead stress condition as compared to normal ones (Awaad et al., 2010).

As methionine is precursor of ethylene, increase in its content is an indication of stress in plant tissues.
(Yang and Adams, 1980). Leaves and fruits under stress condition liberates ethylene (Yang and Hoffman, 1984). In a similar study high level of ethylene was reported in malformed tissues of mango, as compared to healthy ones (Singh and Dhillion, 1990). Similarly (Bains et al., 2003) reported high ethylene level in malformed panicles and shoots bearing them at all developmental stages than healthier ones.

**CONCLUSION**

The data recorded in present study shows that low temperature and high R.H prevails in northern belt of India in the months of February and March during flower initiation and flowering stages in mango. These weather parameters are known to cause malformation disorder in the crop. The analysis of malformed vegetative tissues collected from different northern states revealed rise in the content of MDA and methionine. This is an indicator of stress in plants as MDA is produced due to peroxidation of lipids and methionine being precursor of ethylene leads to its synthesis which is an indicator of stress in plants. Besides this, reduced content of total chlorophyll recorded, indicates the breakdown of membranes due to stressed condition.

**REFERENCES**


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**Table 1**: Meterological data of five states (Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi) of northern India.
Estimation of MDA, Total Chlorophyll and Methionine Content in Relation to Malformation Disorder of Mango (Mangifera indica L.) under Low Temperature and High Relative Humidity Condition Prevailing in Different States of North India

Table 1. Malondialdehyde (MDA) content (µ mol g⁻¹ fw) in malformed and healthy leaf tissues of different mango varieties from different states

<table>
<thead>
<tr>
<th>S. No</th>
<th>Variety</th>
<th>Bihar</th>
<th>Jharkhand</th>
<th>Uttar Pradesh</th>
<th>Uttarakhand</th>
<th>Delhi</th>
</tr>
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<tbody>
<tr>
<td></td>
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<td>Malformed</td>
<td>Healthy</td>
<td>Malformed</td>
<td>Healthy</td>
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<tr>
<td>1.</td>
<td>Annapali</td>
<td>0.436</td>
<td>±0.016</td>
<td>0.114</td>
<td>±0.002</td>
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<td>2.</td>
<td>Dasheri</td>
<td>0.424</td>
<td>±0.017</td>
<td>0.119</td>
<td>±0.002</td>
<td>0.412</td>
</tr>
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<td>3.</td>
<td>Langra</td>
<td>0.435</td>
<td>±0.003</td>
<td>0.120</td>
<td>±0.003</td>
<td>0.417</td>
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<tr>
<td>4.</td>
<td>Chau'a</td>
<td>0.472</td>
<td>±0.019</td>
<td>0.117</td>
<td>±0.008</td>
<td>0.450</td>
</tr>
<tr>
<td>5.</td>
<td>Bombay Green</td>
<td>0.450</td>
<td>±0.004</td>
<td>0.108</td>
<td>±0.002</td>
<td>0.461</td>
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<tr>
<td>Mean</td>
<td></td>
<td>0.443</td>
<td>±0.016</td>
<td>0.116</td>
<td>±0.003</td>
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Fig 1: Malondialdehyde content (µ mol g⁻¹ fw) in malformed and healthy leaves of different mango varieties from different states
Estimation of MDA, Total Chlorophyll and Methionine Content in Relation to Malformation Disorder of Mango (Mangifera indica L.) under Low Temperature and High Relative Humidity Condition Prevailing in Different States of North India

Table 2: Total chlorophyll content (mg g⁻¹ fw) in malformed and healthy leaf tissues of different mango varieties from different states

<table>
<thead>
<tr>
<th>S. No</th>
<th>Variety</th>
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<th>Bihar Healthy</th>
<th>Jharkhand Malformed</th>
<th>Jharkhand Healthy</th>
<th>Uttar Pradesh Malformed</th>
<th>Uttar Pradesh Healthy</th>
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<td>±0.10</td>
<td>±0.07</td>
<td>±0.10</td>
<td>±0.07</td>
<td>±0.10</td>
<td>±0.07</td>
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<td>Amrapali</td>
<td>2.34</td>
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<td>2.51</td>
<td>3.12</td>
<td>2.48</td>
<td>3.12</td>
<td>2.35</td>
<td>3.12</td>
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<td>2</td>
<td>Dasheri</td>
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<td>2.74</td>
<td>3.32</td>
<td>2.63</td>
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<td>3.15</td>
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<td>3</td>
<td>Langra</td>
<td>2.97</td>
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<td>2.15</td>
<td>3.11</td>
<td>2.72</td>
<td>3.11</td>
<td>2.75</td>
<td>3.18</td>
<td>2.57</td>
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<td>4</td>
<td>Chausa</td>
<td>2.48</td>
<td>3.30</td>
<td>2.60</td>
<td>3.35</td>
<td>2.80</td>
<td>3.44</td>
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<td>Bombay Green</td>
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<td>3.11</td>
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<td>±0.04</td>
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<tr>
<td>CD at 5%</td>
<td>±0.09</td>
<td>±0.14</td>
<td>±0.08</td>
<td>±0.13</td>
<td>±0.28</td>
<td>±0.10</td>
<td>±0.15</td>
<td>±0.04</td>
<td>±0.07</td>
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</table>

Fig 2: Total chlorophyll content (mg g⁻¹ fw) in malformed and healthy leaves of different mango varieties from different states
Table 3: Methionine content (µg g⁻¹ fw) in malformed and healthy leaf tissues of different mango varieties from different states

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Variety</th>
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<td>Malformed</td>
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<td>1</td>
<td>Amrapali</td>
<td>94.35 ±2.20</td>
<td>62.65 ±1.12</td>
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<td>62.11 ±0.87</td>
<td>96.00 ±2.46</td>
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<td>Dushehari</td>
<td>99.10 ±4.07</td>
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<td>63.72 ±0.89</td>
<td>95.25 ±2.08</td>
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<td>Langra</td>
<td>100.72 ±1.65</td>
<td>64.72 ±0.89</td>
<td>99.95 ±2.54</td>
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<td>100.75 ±2.34</td>
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<tr>
<td>4</td>
<td>Chausa</td>
<td>95.72 ±0.69</td>
<td>63.89 ±1.39</td>
<td>100.21 ±2.66</td>
<td>63.61 ±1.38</td>
<td>99.45 ±1.38</td>
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<td>5</td>
<td>Bombay Green</td>
<td>93.54 ±1.74</td>
<td>62.70 ±1.43</td>
<td>95.33 ±2.64</td>
<td>62.74 ±1.83</td>
<td>95.26 ±1.91</td>
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<td>97.65 ±1.36</td>
<td>63.87 ±1.34</td>
<td>98.07 ±2.63</td>
<td>63.61 ±1.41</td>
<td>97.34 ±1.41</td>
</tr>
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</table>

Table 3: Methionine content (µg g⁻¹ fw) in malformed and healthy leaf tissues of different mango varieties from different states.

Fig 3: Methionine content (µg g⁻¹ fw) in malformed and healthy leaves of different mango varieties from different states.