Impact of Tea Mosquito Infestation on Endogenous Hormones of Tea (Camellia sinensis L.)

ABSTRACT: Young shoots of tea plants, beverage crop of significance of North East India, are infested by Tea Mosquito Bug (Helopeltis theivora Waterhouse, Hemiptera) leading to enormous crop loss and delayed flushing. This paper aims to find out the variation of plant hormones in non-infested and infested leaves of tea plants and its influence on growth behaviour. Endogenous hormones [auxin (IAA), abscisic acid (ABA) and gibberellic acid (GA3)] play critical roles in dormancy and flushing of tea plants. Helopeltis infestation significantly regulated the content of hormones (IAA and GA3) as well as stress hormone (ABA).

Key words: Tea, Tea Mosquito Bug, Auxin, Abscisic Acid, Gibberellic Acid

Tea plant cultivated in India is a beverage crop having worldwide significance. Tea mosquito bug, Helopeltis theivora Waterhouse (Hemiptera: Miridae) has been a major pest of tea in North East India causing considerable crop loss1 (Fig.1). About 80% area of the tea plantation in North East India is affected by this pest which reduces 10 – 50% productivity2. The average mean shoot infestations during 2005 and 2006 were 24 and 21% respectively3. Damage by Helopeltis is of two kinds: i) direct loss of crop due to damaged leaf and shoots and ii) acute debilitation of the bushes leading to die-back of productive biomass and delayed flushing of infested shoots.

Helopeltis attack is severe during the months of May-September, with high temperature and rainfall. Due to curling and drying up of the leaves severe loss of biomass occurs after Helopeltis attack. Activities of phenylalanine ammonia-lyase and content of polyphenols generally decreased as a result of insect attack. HPLC analysis of catechins revealed a decrease in some of the catechins in the infested leaves4. Phloem-feeding insects provide additional challenge to plants as they deplete photosynthates, vector viruses, and introduce chemical and/or protein effectors that alter plant defense signaling, infestation symptoms and plant development5 and cause heavy losses in agriculture and horticulture6.

After the insect attack on the young leaves and buds, the growth of buds (flushing) stops and thereby the formation of axillary buds delayed. Suspension of growth after Helopeltis attack may be the consequence of hormonal imbalance.

In plants, auxin is essential for growth and development. Often present as indole-3-acetic acid (IAA), it plays a critical role in regulating cell growth, division and differentiation, and on a gross morphological scale auxin clearly impacts apical dominance, root elongation, lateral root formation and many other processes7,8. The gibberellic acids (GAs), a group of diterpenoid compounds with phytohormone activity, affect various stages of plant development, including seed germination, stem elongation, root growth, flowering and pollen tube elongation7,9. The hormone abscisic acid (ABA) is another prominent regulator of seed germination that also enables plants to respond to abiotic stresses such as drought. ABA can directly affect ion transport in guard cells to alter stomatal aperture rapidly in response to changing water availability10. In tea, a detailed study on growth and dormancy has been made different seasons indicating fluctuations of endogenous hormones11,12,13,14.

This study aims to find out the contents of endogenous hormones, viz. abscisic acid (ABA), gibberellic acid (GA3) and indole-3-acetic acid (IAA) in tea leaves infested by herbivorous insect, Helopeltis theivora.

Materials and Methods: TV1 is a standard tea clone and S.3A/3 and T.3E/3 are quality clones widely cultivated in tea gardens. Leaf samples of 10 g each were taken from both non-infested (without any infestation spots on the leaf by insect) and infested plants of three clones (TV1, S.3A/3 and T.3E/3) grown in the growth habit plots of Tocklai Experimental Station, Jorhat, Assam, India (26°47’N, 94°13’E), under identical conditions. The materials were homogenized with 80% chilled methanol which was centrifuged at 4°C under 10000 rpm and filtered. The residues were again homogenized, centrifuged and filtered. The total volume of the filtrate was reduced to one third where 15ml of saturated lead acetate solution was added and kept at 4°C for 4 hours. After centrifugation, pH of the filtrate was adjusted to 3.0. The extract was again centrifuged and filtered. The filtrate was extracted 3 times with ethyl acetate and the fraction was reduced to one third. The filtrate was extracted 4 times with 1% NaHCO3.
and pH was adjusted to 3.0. It was further extracted with ethylacetate (99.5%). The filtrate was evaporated to dryness. The dried sample was dissolved in 10ml methanol of HPLC (High Performance Liquid Chromatography) grade and filtered. Charcoal powder was added and kept for 4 hours and filtered. The solution was finally filtered through micro mate interchangeable hypodermic syringe before it was injected to the HPLC 15,16. Auxin (IAA), abscisic acid (ABA) and gibberellic acid (GA 3) were detected using HPLC (Waters 2487 Dual λ Absorbance Detector, USA) and the following specifications were followed. All the experiments were replicated thrice.

**Results and Discussion**

**ABA**

ABA content was higher in T.3E/3 than TV1 and S.3A/3 (Fig.2A) in both non-infested and infested shoots. Percentages of increase of ABA in infested shoots of TV1 (18.54) and T.3E/3 (14.70) were much higher than S.3A/3 (8.00) (Table 1).

ABA is known as a dehydration stress hormone and interferes with defenses against pathogens17. ABA enhances the defense through at least two independent mechanisms, viz. callose priming and regulation of defense gene expression through activation of Jasmonic Acid biosynthesis18. In tomato, loss of ABA reduced resistance against caterpillars19. ABA content increased in *Populus* after infestation by herbivorous insect20. The contents of ABA increase in all the tea clones infested by *Helopeltis*. By increasing the level of ABA, the tea clones try to scavenge the stress conditions.

*Helopeltis* infestation was much less in S3A/3, a low ABA synthesizing clones than in clones T3E/3 and TV1 which are high

<table>
<thead>
<tr>
<th></th>
<th><strong>Abscisic acid</strong></th>
<th></th>
<th><strong>Gibberellic acid</strong></th>
<th></th>
<th><strong>Indole -3- acetic acid</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Micro Bondapack C-18 reverse phase (300×4 mm i.d)</td>
<td>Column</td>
<td>Micro Bondapack C-18 reverse phase (300×4 mm i.d)</td>
<td>Column</td>
<td>Novapack ODS C 18 reverse phase (150×3.5 mm i.d)</td>
</tr>
<tr>
<td>Wavelength</td>
<td>UV detector: 254nm</td>
<td>Wavelength</td>
<td>UV detector: 206nm</td>
<td>Wavelength</td>
<td>Fluorescence detector: Excited 280nm and Emission 350nm</td>
</tr>
<tr>
<td>Run time (minutes)</td>
<td>15</td>
<td>Run time (minutes)</td>
<td>10</td>
<td>Run time (minutes)</td>
<td>15</td>
</tr>
<tr>
<td>Flow rate (mL min⁻¹)</td>
<td>1</td>
<td>Flow rate (mL min⁻¹)</td>
<td>1.8</td>
<td>Flow rate (mL min⁻¹)</td>
<td>1</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>55% methanol in 1% acetic acid (HPLC grade)</td>
<td>Mobile phase</td>
<td>20% methanol in 0.01M H₃PO₄ (HPLC grade) and adjust pH to 2.3 with KOH</td>
<td>Mobile phase</td>
<td>40% methanol in 0.1% acetic acid (HPLC grade)</td>
</tr>
</tbody>
</table>

Calculation:

Growth hormone (GH) quantification

\[ GH = \frac{(A \times B \times X \times C)}{D} \]

where A= Standard (ppm); B= Sample injected (µL); C=Sample fresh weight (g); D= Peak area of the sample; X=Standard peak area

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Fig.1 Infestation by *Helopeltis*. (A) Sucking lesions on young leaf (B) Infestation on second and third leaves (C) Portion of bush showing infestations (D) An infested bush which is non productive
ABA synthesizer (Fig. 2 A). In non-infested tomato plants, ABA levels are normally higher in leaves of a susceptible cultivar than a tolerant cultivar to the carmine spider mite. Thus the lower production of ABA enhanced the resistance of the clone to *Helopeltis*.

**GA$_3$**

GA$_3$ content was higher in non-infested as well as in infested shoots in T3E/3 than TV1 and S3A/3 (Fig. 2B). Percent increase of GA$_3$ in infested shoots of S.3A/3 (168.5) was higher than both TV1 (71.4) and T.3E/3 (67.6) (Table 1).

**TABLE 1. Percentage of hormone increase (+) or decrease (-) in infested leaves as compared to healthy leaves of tea (Camellia sinensis L.)**

<table>
<thead>
<tr>
<th>Clones</th>
<th>ABA</th>
<th>GA$_3$</th>
<th>IAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>TV1</td>
<td>+18.54</td>
<td>+71.40</td>
<td>-55.90</td>
</tr>
<tr>
<td>S.3A/3</td>
<td>+8.00</td>
<td>+168.50</td>
<td>-50.40</td>
</tr>
<tr>
<td>T.3E/3</td>
<td>+14.70</td>
<td>+67.60</td>
<td>-56.90</td>
</tr>
</tbody>
</table>

Gibberellic acids are known for their role in developmental processes. GA$_3$ contents increased after infestation by *Helopeltis* in all the tea clones. On injecting GA$_3$ and IAA in the dormant tea plants in winter, it was observed that GA$_3$ resulted in early flushing while IAA had no effect on the dormant tea plants. Red spider mite infested plum tree has higher level of gibberellic acid content and lower level of auxin than the non-infested one.

**IAA**

IAA content was higher in non-infested as well as of infested shoots in TV1 than S3A/3 and T3E/3 (Fig. 2C). Percentages of decrease of IAA in infested shoots of T.3E/3 (56.90) and TV1 (55.90) were higher than S.3A/3 (50.40) (Table 1).

**TABLE 2. Ratio of IAA:ABA in non-infested and infested leaves of tea (Camellia sinensis L.) clones by Helopeltis theivora**

<table>
<thead>
<tr>
<th>Clones</th>
<th>Non-infested</th>
<th>Infested</th>
</tr>
</thead>
<tbody>
<tr>
<td>TV1</td>
<td>0.065</td>
<td>0.024</td>
</tr>
<tr>
<td>S.3A/3</td>
<td>0.059</td>
<td>0.027</td>
</tr>
<tr>
<td>T.3E/3</td>
<td>0.031</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Auxin is synthesized in young expanding leaves at the shoot apex and is actively transported down the plant in the polar transport stream. *Helopeltis* attack on the axillary vegetative buds and young leaves of tea resulted in decrease in auxin content. It was noticed that the sprouting of axillary buds were delayed in all the clones. In tea, biosynthesis of auxin was found abundant in the first internode and unopen buds of growing two and a bud. Thus the *Helopeltis* infestation is likely to affect the site of auxin biosynthesis. However, decrease in auxin may not be the main cause of delaying the sprouting of axillary bud. Morris *et al*. showed that a change in IAA content could occur in the stem without stimulating bud outgrowth.

A comparison of IAA:ABA ratio in infested and non-infested tea leaves are shown in Table 2. Relatively higher ratio of IAA:ABA was observed in TV1 and S.3A/3 than T.3E/3 when leaves were non-infested. However, an opposite observation was documented in the case of infested shoots where the ratio in S.3A/3 was found to be higher than in TV1 and T.3E/3. In infested shoots of TV1, the IAA:ABA ratio was two-fold higher than T3E/3. Gensheng *et al* showed...
that the growth of tea shoots was influenced by the ratio of IAA and ABA.

**Conclusion**

Variations of ABA, GA₃ and IAA contents in non-infested and infested tea leaves by *Helopeltis* were observed in this study. Early recovery after Helopeltis attack in S.3A/3 compared to the other two clones may be due to the less increase of ABA and less decrease of IAA coupled with more increase of GA₃. The percent increase of GA₃ which is very high in S.3A/3 compared to the other clones is an important factor for early flushing after Helopeltis infestation. GA₃ may be essential to overcome the biotic stress and for subsequent growth and development of tea plants.

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