INVESTIGATIONS INTO THE ORGANIC ACID METABOLISM OF TAMARINDUS INDICA LINN.

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Received March, 25 1953
[Communicated by Prof. J. Venkateswarlu, D.Sc., Ph.D. (Cantab.), F.A.Sc.]

Although Benjamin Heyne (1815) and Mayer (1878) were the earliest to observe that leaves of succulent plants like Bryophyllum exhibit diurnal fluctuation in acidity, the acid content being highest in the morning and lowest in the evening, it is only since the publication of the monumental work of Bennet-Clark (1933) that the phenomenon has come to be widely known as Crassulacean metabolism. Since this publication a large number of workers investigated the acid metabolism of succulent plants and also of a few non-succulent plants. Notable among these are the studies of Vickery, Pucher and Co-workers [Vickery and Pucher (1940); Allsopp (1937); Bennet-Clark and Woodruff (1937); Thomas (1949); Bonner and Bonner (1948); and Somers (1951)]. The work on acid metabolism has been reviewed by Bennet-Clark (1937), Vickery and Pucher (1940) and Bennet-Clark (1949). In his review of 1933 Bennet-Clark expressed the view that all those non-succulent plants which possess high enough concentrations of organic acids also exhibit a Crassulacean metabolism. It has, however, been found in recent years that the behaviour of some non-succulent plants like rhubarb, Nicotiana and Fagopyrum is not entirely in conformity with this type of metabolism (Pucher, Wakeman and Vickery, 1938; Vickery and Pucher, 1939; Pucher, Wakeman and Vickery, 1939). Investigations have now been extended to Tamarindus indica Linn., a plant with non-succulent leaves and showing accumulations of acids both in the leaves and in the fruits, with a view to study how far the acid metabolism of the plant compares with the Crassulacean type. The present paper deals with the titratable acidity changes with age, diurnal and seasonal fluctuations in titratable acidity, pH changes with age and the buffering capacity of the leaf sap of Tamarindus indica Linn.

Materials and Methods

A Tamarindus tree growing in the Andhra University campus at Waltair provided the material for the investigation. It is a healthy tree growing luxuriantly, flowering and fruiting regularly. Vegetative buds were marked as they came up, by means of date labels. As they begin to open and grow,
the leaves that are found at the tip and that come out first are referred to as Group I, then the next bigger leaves as Group II and so on. The age of the leaves increases from the tip to the base of the twig and the leaves from the youngest to the oldest could be roughly divided into 4 or 5 groups each with its own characteristic T.A.N. value as will be seen later. Random samples are taken of leaves in the various age groups.

There exists a difference of opinion among the various authors regarding the relative usefulness of titratable acidity and total acidity determinations in studies of the organic acid metabolism of green plants. Bennet-Clark (1933) believes that changes in titratable acidity give a fairly correct indication of the changes in total acidity as plants do not contain any bases in sufficiently large quantities to neutralise the acids. Moreover, titratable acidity determinations can be made with a greater degree of accuracy and in a shorter time than estimations of total acidity. On the other hand, Vickery and Pucher (1940) remark that "the day is now past when the results of a simple titration can be calculated in terms of malic acid or citric acid and interpreted in any meaningful way". Thomas (1947) says "Nevertheless, simple titration may continue to give suggestive information." As recently as 1949 Thomas working on Bryophyllum relied entirely on titratable acidity. Somers (1951) working on Kalanchoe daigremontiana measured only titratable acidity and pH. As such in the present studies observations have been made of titratable acidity and pH in the leaves of Tamarindus.

For measuring titratable acidity of the leaves, about 1 gm. of the material was accurately weighed and ground in a mortar, then boiled, filtered and the filtrate titrated against 1/50 N Na(OH). Brom Thymol Blue (B.D.H. Parstains label) was used as the indicator. It shows the end point in the pH range 6-7.6 and is yellow in acid medium. The end point is indicated by the turning of the yellow colour to blue. The filtrate was free from chlorophyll and no interference was met with during titration.

Bennet-Clark (1933) expressed titratable acidity in terms of mg. equivalent per 100 gm. fresh weight of tissue. He used N/50 Na(OH) for his titrations. Thomas (1949), on the other hand, expressed titratable acidity in terms of the Titratable Acid Number (T.A.N.) which he defined as the amount in c.c. of 1/10 N Na(OH) required to neutralise an extract from 100 m.g. of tissue. In the present investigation the T.A.N. notation was adopted with slight modifications, namely, instead of 1/10 N, 1/50 N Na(OH) was used and instead of 100 mg., 1,000 mg. of the leaf tissue were used. The changes were necessitated by the relatively low quantities of acid present in Tamarindus.
$pH$ of the sap extracted by crushing the leaves was measured by means of the Beckman $pH$ meter (laboratory model G).

I. Titratable Acidity of Leaves

(a) Variation with age.—The T.A.N. values of leaves of different ages were determined and given in Table I. It is observed that the titratable acidity is very low in the youngest leaves and increases rapidly with age. Acidity falls again in the oldest leaves.

Table I

Variation in the T.A.N. with the ageing of the leaves

<table>
<thead>
<tr>
<th>Position of leaf from tip</th>
<th>1</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>T.A.N.</td>
<td>19.8</td>
<td>25.5</td>
<td>30.2</td>
<td>24.5</td>
<td>22.8</td>
</tr>
</tbody>
</table>

(b) Diurnal fluctuations in acidity.—In studies of the titratable acidity of leaves at various hours during the day, the leaves were divided into different age groups so that variation due to age might not mask the diurnal fluctuations and the diurnal fluctuations in the different age groups were studied separately. The T.A.N. values of leaves of the different age groups at various hours during the day have been recorded in Table II. The age groups are numbered from I to IV starting from the youngest leaves at the tip to the older ones towards the base of the twig.

Table II

T.A.N. Values of leaves of different age groups at various hours during the day

**GROUP I**

<table>
<thead>
<tr>
<th>Time</th>
<th>7 a.m.</th>
<th>10 a.m.</th>
<th>1 p.m.</th>
<th>4 p.m.</th>
<th>7 p.m.</th>
<th>10 p.m.</th>
<th>1 a.m.</th>
<th>4 a.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>T.A.N.</td>
<td>17.4</td>
<td>18.3</td>
<td>18.1</td>
<td>17.65</td>
<td>17.5</td>
<td>17.85</td>
<td>17.55</td>
<td>17.55</td>
</tr>
</tbody>
</table>

**GROUP II**

<table>
<thead>
<tr>
<th>Time</th>
<th>7 a.m.</th>
<th>10 a.m.</th>
<th>1 p.m.</th>
<th>4 p.m.</th>
<th>7 p.m.</th>
<th>10 p.m.</th>
<th>1 a.m.</th>
<th>4 a.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>T.A.N.</td>
<td>24.5</td>
<td>26.2</td>
<td>25.5</td>
<td>25.3</td>
<td>25.2</td>
<td>26.2</td>
<td>25.7</td>
<td>26.4</td>
</tr>
</tbody>
</table>
**Organic Acid Metabolism of Tamarindus indica**

GROUP III

<table>
<thead>
<tr>
<th>Time</th>
<th>7 a.m.</th>
<th>10 a.m.</th>
<th>1 p.m.</th>
<th>4 p.m.</th>
<th>7 p.m.</th>
<th>10 p.m.</th>
<th>1 a.m.</th>
<th>4 a.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>T.A.N.</td>
<td>28.3</td>
<td>28.3</td>
<td>28.3</td>
<td>27.8</td>
<td>28.6</td>
<td>27.7</td>
<td>27.7</td>
<td>28.2</td>
</tr>
</tbody>
</table>

GROUP IV

<table>
<thead>
<tr>
<th>Time</th>
<th>7 a.m.</th>
<th>10 a.m.</th>
<th>1 p.m.</th>
<th>4 p.m.</th>
<th>7 p.m.</th>
<th>10 p.m.</th>
<th>1 a.m.</th>
<th>4 a.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>T.A.N.</td>
<td>21.5</td>
<td>22.5</td>
<td>21.72</td>
<td>21.2</td>
<td>21.9</td>
<td>22.35</td>
<td>22.0</td>
<td>22.1</td>
</tr>
</tbody>
</table>

These observations were made on a bright sunny day in the month of September. These were repeated on several consecutive days of the same month for 2 years and almost identical values were obtained in the various age groups. It will be seen that there is little diurnal fluctuation, the maximum difference between the lowest and highest values in a day never exceeding 6%. In those plants in which Crassulacean metabolism was recorded the variation in acid content between day and night values was usually high, sometimes reaching as high a value as 1260% (Kraus, 1883).

It was generally observed by previous workers that although there may not be any considerable acidity fluctuation in the youngest leaves, the older leaves definitely showed marked diurnal fluctuation (Bennet-Clark, 1933). In the present study, however, no diurnal fluctuations were observed in any of the age groups. The slight variations on either side of the average characteristic of a particular group might be attributed to chance variations.

(c) **Seasonal fluctuations.**—T.A.N. was determined for leaves of Group I in the various months of the year and recorded in Table III.

**TABLE III**

*T.A.N. values of Group I leaves in various months of the year*

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum</td>
<td>18.3</td>
<td>18.5</td>
<td>21.3</td>
<td>23.9</td>
<td>28.0</td>
<td>26.1</td>
<td>23.0</td>
<td>20.0</td>
<td>17.0</td>
<td>16.8</td>
<td>17.3</td>
<td>18.0</td>
</tr>
<tr>
<td>Minimum</td>
<td>17.4</td>
<td>16.0</td>
<td>20.0</td>
<td>24.5</td>
<td>27.1</td>
<td>24.2</td>
<td>21.3</td>
<td>18.6</td>
<td>16.0</td>
<td>16.5</td>
<td>16.9</td>
<td>18.2</td>
</tr>
</tbody>
</table>

The T.A.N. values are highest in the winter months of December, January and February when the lowest temperatures of the year are obtained and when the period is preparatory to flowering. On the other hand, under
temperate conditions Allsopp (1937) working on the rhubarb plant found the greatest accumulation of acids in the green parts of the rhubarb plant during the summer months while only slight increase in the acid content is observed in the rhizome in the winter months. Thomas (1947) explained these results by assuming that acids are probably produced and accumulated in the green shoots in the summer and are later translocated to the rhizome. Bennet-Clark (1930) also observed highest acidity in the summer months in *Sedum praelatum* and low values in the winter months. The summer is the best growing period in the temperate conditions and so the greatest accumulation is found in that season there while in this tropical plant *Tamarindus indica* the highest acidity is observed in late winter just before flowering which takes place in April and May.

It is also of interest to note that in *Tamarindus* no diurnal variation in titratable acidity has been observed during the course of a day in any month of the year in any of the age groups.

**II. pH of the Leaf Sap**

*(a) Variation with age.*—The *pH* of the sap of leaves of the various age groups was determined and shown in Table IV. The *pH* is seen to decrease with increasing age. Only in the oldest leaves a slight rise in *pH* is observed. These changes are thus concurrent with corresponding changes in titratable acidity with the ageing of the leaf.

<table>
<thead>
<tr>
<th>Age group</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>pH</em></td>
<td>3.5</td>
<td>3.48</td>
<td>3.3</td>
<td>3.12</td>
<td>3.2</td>
</tr>
</tbody>
</table>

*(b) Buffering action.*—Titration curve is drawn for leaves of Group III (Fig. 1). It will be seen that fully mature leaves of Group III have a high buffering capacity.

**DISCUSSION**

*Tamarindus indica* with a *pH* of 3.1-3.5 falls in the acid group of plants in Small's (1946) scheme of classification. From the titration curve it will be seen that the buffering capacity of the fully grown leaves is of a high order. Sinclair and Eny (1947) working on the buffer properties of citrus peels found that the juice of the pulp has a greater buffering capacity than the peel. So they concluded that in the presence of organic acids the buffering capacity
The data presented in this investigation show that Tamarindus exhibits some of the features of plants having Crassulacean metabolism, namely, (1) increasing titratable acidity and decreasing pH with ageing and (2) seasonal variation in acidity. However, there is one important difference—the absence of diurnal fluctuation in acidity. A number of plants have recently been brought to light which do not exhibit diurnal fluctuation in acidity. Thomas (1949) did not observe any diurnal fluctuation in the leaves of Geranium pratense which according to Bennet-Clark’s list (1933) showed 700% increase in titratable acidity overnight. Bonner and Bonner (1948) failed to observe
the Crassulacean type of metabolism in *Mysembryanthemum edule* and a species of *Sedum* in both of which they found an accumulation of large amounts of acid under all conditions. Bennet-Clark (1933) mentioned that various species of *Mysembryanthemum* were shown to exhibit increase as well as decrease of acidity during night by several authors. *Sedum præeltum* was hitherto regarded as a plant showing Crassulacean metabolism. Non-succulent plants like rhubarb, *Nicotiana* and *Fagopyrum* (Pucher, Wakeman and Vickery, 1938; Vickery, Pucher, 1939; and Pucher, Wakeman and Vickery, 1939) were also found to show no Crassulacean metabolism and the real nature of their metabolism is still obscure. Therefore, it seems necessary to devote more attention to this category of plants than has hitherto been done.

Bennet-Clark (1933) suggests that the absence of diurnal fluctuation may be due to either of two reasons; the enzyme system responsible for acid production and depletion is very weak or the rate of depletion of acids is perhaps as fast as that of their formation.

One of the external factors that influences the rate of acid formation and depletion is temperature. Low temperatures generally favour acid accumulation while high temperatures bring about acid depletion (Bennet-Clark, 1933). More recently Somers (1951) came to a similar conclusion. The present study has revealed that *Tamarindus* has greatest titratable acidity in winter months and the lowest in summer months. It is therefore evident that fluctuations in temperature have the same kind of effect on the acid metabolism of *Tamarindus* as of other plants previously recorded. Besides the specific nature of the acid metabolism of the plant it is thus possible that the absence of marked diurnal fluctuations in temperature in Waltair in the different months of the year is also partly responsible for the absence of diurnal fluctuations in acidity.

**Summary**

The titratable acidity and *pH* of leaf sap of *Tamarindus indica* Linn. have been determined under various conditions. The T.A.N.'s of leaves of different ages have been determined and they show an increase with increasing age until in the oldest leaves again there is a slight fall. The T.A.N. values of different age groups have been determined at various hours during the day and it has been found that there is no diurnal fluctuation in acidity in any of the groups. The T.A.N. of leaves of Group 1 has been determined in the various months of the year. The titratable acidity is highest in the winter months and lowest in summer months. The *pH* shows
a decrease with increasing age until in the oldest leaves again it increases with age. The buffering capacity of the leaf sap is of a high order.

ACKNOWLEDGEMENTS

Our thanks are due to Prof. G. N. Rangaswami Ayyangar and Prof. J. Venkateswarlu for their kind encouragement.

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*Original not seen by the authors.