

Table 1. Components of the organic acid system of the leaves of *Bryophyllum calycinum* at different hours of the day

Time	Malic Acid	Isocitric Acid	Oxalic Acid	Succinic Acid
7 a.m.	+++	++	0	0
10 a.m.	+++	++	0	0
1 p.m.	++	+++	0	0
4 p.m.	+	+++	+	0
7 p.m.	+	+++	++	-
10 p.m.	++	++	-	-

+++ Very prominent; ++ prominent; + present; - in traces; 0 not found.

minor role. The organic acid composition of the young leaves of *Bryophyllum calycinum* at different hours of the day are given in Table 1.

**Results and Discussion.** From Table 1 it is clear that the malic and isocitric acids are the major components of the organic acid system of the young leaves of *Bryophyllum calycinum*, while oxalic and succinic play only a minor role. It is also evident that the acid composition varies at different hours of the day. At 7 a.m. only two acids appear indicating that acidity in the morning is chiefly due to the malic and isocitric acids of which the former is more dominant than the latter. This composition remains unchanged upto 10 a.m. However at 1 p.m. malic acid shows decrease while the isocitric shows increase, thus indicating rapid consumption of the former during this period. At 4 p.m. malic acid decreases still further and isocitric remains stationary. At the same time a new acid appears on the chromatogram namely, the oxalic acid. This new acid does not move properly in the solvent system employed but spreads right upto malic acid and therefore could not be reproduced properly in the photograph.

At 7 p.m. the situation remains the same as at 4 p.m. except that oxalic acid shows still further increase and succinic acid appears for the first time. At 10 p.m. malic acid increases, isocitric decreases and oxalic totally disappears. However succinic remains the same as a trace acid.

In conclusion it can be said that by the chromatographic technique malic acid is found to be the chief component in the morning which decreases in the afternoon, reaches minimum in the evening, while the isocitric acid shows accumulation with the advance of the day and is the chief component of the acid fraction by evening. Oxalic and succinic appear merely in small amounts and their presence is seen on the chromatogram in the evening extract at 4 p.m. only when the stock of malic acid is much depleted. Therefore our observations though qualitative in nature, confirm the results obtained by PUCHER *et al.*<sup>5)</sup> who employed analytical methods.

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Department of Botany, Institute of Science, Bombay, India

F. R. BHARUCHA and G. V. JOSHI

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#### Identification of Organic Acids in the Leaves of *Bryophyllum Calycinum* by Paper Chromatography

The investigations of BENNET-CLARK<sup>1)</sup> and THOMAS<sup>2)</sup> in the United Kingdom, PUCHER and VICKERY<sup>3)</sup> in America and WOLF<sup>4)</sup> in Germany show that succulents in general exhibit diurnal fluctuations in the acid contents of their photosynthetic parts and thereby exhibit a special type of metabolism known as the Crassulacean type of metabolism, of which *Bryophyllum calycinum* is the classical example. While BENNET-CLARK<sup>1)</sup> and THOMAS<sup>2)</sup> mostly relied on volumetric methods to demonstrate these changes in acid contents, PUCHER and his coworkers<sup>3)</sup> developed analytical methods to estimate individual acids by which they showed that it is the malic acid which takes the most important part in these fluctuations while the isocitric follows an irregular course. In the present work an attempt has been made to show these changes in the acid contents of the leaves of *Bryophyllum calycinum* SALISB. by employing the chromatographic technique. This technique being a sensitive one it is possible to detect acids present even in tracer amounts; moreover a permanent record in the form of a photograph of the acid composition at various hours of the day can be obtained.

**Method.** The organic acids of the young leaves of *Bryophyllum calycinum* were extracted in neutral 80% ethanol at intervals of three hours from 7 a.m. to 10 p.m. and the aliquots containing equal total titratable acidity were spotted at six different places on circular paper chromatograms of 35 cm. diameter. The chromatogram was then developed and sprayed by the method of BARNABAS and JOSHI<sup>6)</sup>. It may be mentioned that as the isocitric and citric acids have identical  $R_f$  values in the solvent system employed, the bands of these acids merge and show as one band. This one band will be referred to henceforth as the band of the isocitric acid because analytical investigations of PUCHER *et al.*<sup>3)</sup> have shown that the isocitric acid is the major acid of the leaves of *Bryophyllum calycinum* while citric acid plays only a