photosynthesis of the previous day. It also reveals that under tropical conditions highest accumulation of acids is in winter while it is in summer in temperate countries.

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STUDIES IN CRASSULACEAN METABOLISM IN *ALOE VERA* LINN

B-29

By

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Benjamin Heyne (1815) reported from India that the leaves *Bryophyllum calycinum* taste sour in mornings, tasteless in afternoons, and are bitterish in evenings. Due to investigations undertaken by Bennet-Clark (1933) and Thomas (1949) in England, Pucher, Vickery and co-workers (1947, 1947a, 1947b, 1948 and 1948a) in America, and Wolf (1931, 1937, 1938 and 1939) in Germany it is possible today to offer a possible explanation for this phenomenon.

The above investigators showed that succulents in general with a few exceptions accumulate acids during darkness and lose them when exposed to light. This type of diurnal fluctuation in the acid content of the chlorophyllous tissues has been designated as the "Crassulacean metabolism" because plants belonging to the order Crassulaceæ are classical examples of it. Reasearch during last twenty years shows that Crassulacean metabolism is controlled by light, temperature, soil. humidity and other factors in general which control photosynthesis, It further shows that this metabolism involves accumulation and consumption of acids of the Krebs cycle which is believed to be operating in all plant and animal tissues. This fact has enhanced the importance of Crassulacean metabolism.

In the present work, therefore, an attempt is made to study organic acid metabolism in *Aloe vera*, a succulent member of the Liliaceæ, at a tropical place like Bombay. This plant was selected because previous works (Hempel 1917 & Small 1946) show that there is a wide range of diversity regarding acid metabolism in different species of the genus Aloe.

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Material, Conditions, Methods and Results

Pland material:—*Aloe vera* plants were grown on the specially prepared beds in the college compound. When fully developed, plants reached a height of 1½ft. to 2ft. and developed 12-14 aggregate erect succulent leaves. Only fully developed plants were selected for investigation while very young and old plants were rejected. Crassulacean metabolism is either studied in excised leaves cultured in the laboratories or in the leaves attached to the plants. In our experiments we have followed the latter method and the leaves were plucked just before the investigation began. The results of our preliminary experiments showed that the acid synthesis is a vigorous process in the mature and fully developed leaves while it is a slower one in the tender and basal old ones. Therefore, widest fluctuations in acidity were shown by fully developed leaves i.e. 3rd, 4th and 5th leaves and hence these were selected for the experiments.

Experimental conditions:—As Crassulacean metabolism is controlled by environmental factors it is essential to record the environmental conditions under which the experiments were performed. The garden where the experimental plants were grown was situated in a corner of the college compound where direct sunlight was received for 5 to 6 hours of a day. Bombay being a tropical island it has a warm, moist climate and it receives rains during summer and hence belongs to monsoon lands with monsoon type of climate. The year can be divided into three seasons, the cold, the hot and the rainy. The experiments were carried out in three different seasons. These experiments were repeated three to four times during each season under similar conditions and the average results calculated. T.A.N. was determined at intervals of three hours over a period of 24 hours commencing at 7 a.m.

A brief account of the climatic conditions in three different seasons of Bombay is given below.

(1) *Monsoon*:—The average rainfall for Bombay is 70·0". Out of this, most of the rainfall occurs from June to September. Between the last week of July and the first week of August is the period of heaviest rainfall. During this period of heavy rainfall the sky is cloudy, humidity highest (98%) and temperature fluctuations are the least. Experiments were carried out under such conditions.

(2) *Winter*:—Bombay being a coastal place real winter is hardly even experienced. However towards the end of December and beginning of January the lowest temperature of the year (19°C.) is recorded. During this period sky is very clear, atmosphere less humid (66-74%) and temperature fluctuations more than summer and monsoon. This time of winter was selected for our experiments.

(3) *Summer*:—The first fortnight of May is considered to be the hottest of the year during which temperature rises up to 35°C. During this period of summer experiments were carried out.

Experimental methods:—Titratable acidity (T.A.N.) was determined by the method of Thomas and Beevers (1949). 5 g. of the fresh leaf tissue was sliced before plunging it into 100 ml. of boiling water. Boiling was continued for twenty minutes and then cooled to room temperature. The extract was titrated with N/10 sodium hydroxide with a few drops of phenolphthalein as indicator. The end point was fairly distinct.

In the present investigation, the titrable acid number (T.A.N.) is the number of ml. of decinormal sodium hydroxide required to neutralize the acid containing in the boiled residue and the liquid originating from 100 g. of the leaf tissue. The absolute acid content in a known weight of leaves was calculated and expressed in terms of malic acid as done by Thomas and Beevers (Ibid.)

The results obtained during the three seasons are presented in Table 1. These results are the average of five sets and deviation for the highest values may be ± 5 T.A.N. units and for the lowest ± 3 units.

Table I

Diurnal fluctuations in T.A.N.* in mature leaves of Aloe vera in different seasons

Time	Monsoon		Winter		Summer	
	Temp. in °C	T.A.N.	Temp. in °C	T.A.N.	Temp. in °C	T.A.N.
7 a.m.	26.0	45.5	21.5	86.12	30.0	54.69
10 a.m.	26.5	22.3	26.5	54.56	32.0	29.6
1 p.m.	26.5	17.7	28.0	33.5	35.0	14.5
4 p.m.	27.0	8.1	27.5	9.1	34.0	7.68
7 p.m.	27.0	13.1	26.0	11.12	31.0	13.35
10 p.m.	26.0	27.1	24.0	16.0	30.5	20.92
1 a.m.	26.0	33.02	22.0	41.5	30.5	27.2
4 a.m.	26.0	40.6	22.0	60.15	29.5	37.5
Percentage increase	474%		855%		677%	
Relative humidity	90-98%		66-74%		70-76%	
Maximum Tempefature	27.0		28.0		35.0	
Minimum Temperature	26.0		21.5		29.5	
Diff. in Temperature	1.0		6.5		5.5	

* T.A.N. expressed as the number of ml. of decinormal sodium hydroxide required to neutralize the acid containing in the boiled residue and liquid originating from 100 g. of fresh tissue.

Discussion

From table 1 it is clear that there are distinct seasonal variations in diurnal fluctuations in T.A.N. These seasonal variations can be attributed to prevailing environmental conditions in different seasons. The highest dark acidificaton and the highest percentage increases in T.A.N. are in the winter while the lowest are recorded in the monsoon. In the winter at Bomay conditions are extremely favourable for increased photosynthetic and dark acidification activities. A bright sunny day is followed by a cool night when temperature is bout 22°C. Bonner (1950) has stated that rate of acid accumulation is high at 20°C which, according to him, is probably the optimum condition for Crassulacean metabolism. Due to bright sunny day there is an increase in photosynthetic activity which makes greater amount of starch availble for ultimate production of malic acid at night and prevailing low temperature at that time accelerates this metabolic process.

Even though summer and winter may both have bright sunshine and dry atmosphere acid accumulation is not as high in summer as in winter, because, the higher temperature in the former retards the rate of acidification. The lowest possible figures for acidification were obtained in the monsoon. This is because during this period sky is very cloudy, light feeble and atmosphere humid. Under these conditions the rate of photosynthesis is retarded, the production of starch is lessened and acid production tends to be low. Pucher *et. al.* (1949) found that such conditions slow down the rate of acidification. They therefore felt that cloudy sky and humid atmospheres play an important role in controlling the magnitude of Crassulacean metabolism. Our results in monsoon are in conformity with those of Pucher and coworkers.

The works of Bennet-Clark (1933) and Pucher *et. al.* (1947) suggest that the rate of malic acid formation in succulent leaves when placed in dark is related to the intensity of photosynthesis of the previous day. The figures obtained for seasonal variations in diurnal fluctuations in T.A.N. in the leaves of *Aloe vera* support this hypothesis.

As already mentioned there is a wide range of diversity regarding acid metabolism in the different species of the genus Aloe. Hempel (1917) reports that the percentage increase in titratable acidity during night in *Aloe arborscens* is only two while in *Aloe cymbæfolia* it is 840. This work on *Aloe vera* records percentage increases of 474, 677 and 855 for monsoon, summer and winter respectively. Even the lowest of these is higher than the figures of Hempel. Small (1946) found that

the cellular pH of *Aloe variegata* is above 5.2 thereby suggesting sluggish acid metabolism of the succulent. We found that the cellular pH in *Aloe vera* is near about 4·2 suggesting that acid metabolism in *Aloe vera* is more vigorous than that of *Aloe variegata*. Hempel and Small have recorded these readings under temperate conditions while we have carried out investigations under tropical conditions and these differences may be due to influence of tropical conditions.

Another interesting fact observed from the present investigation is that under tropical conditions highest accumulation in acidity is in winter while it is not so in temperate countries. Allsop (1937) working in England found the greatest accumulation of acids in the rhubarb plant during summer. Bennet-Clark (1933) working in the same country observed highest acidity during the same season in *Sedum prælatum* and low values in winter. Kraus (1883) investigating under temperate conditions recorded highest percentage increase in T.A.N. in Bryophyllum leaves in June while Thomas and Beevers (1949) working at Newcastle report high accumulation for the same species in May. All these investigations were carried out in Europe where summer is the best season conducive to growth. In the present investigation it was found that highest accumulation of acids is seen in winter. Sheshgiri and Sastri (1953) working under tropical conditions in this country found that highest values in T.A.N. for the leaves of *Tamarindus indicus* are obtained in December, January and February. Therefore it can be concluded that under tropical conditions late winter time is best for acid accumulation while it is summer under temperate ones.

Summary

A study in Crassulacean metabolism in *Aloe vera* has been made at Bombay. It was found that this succulent shows both aspects of Crassulacean metabolism namely (1) increase in acidity with maturity of leaves and its fall with the onset of senescence (2) diurnal fluctuations in T.A.N. in chlorophyllous parts. The magnitude of diurnal fluctuations varies from season to season and it is highest in winter and lowest in monsoon. These seasonal variations can be attributed to prevailing environmental factors. The results obtained for *Aloe vera* are higher than those recorded for *Aloe cymbæfolia*, *Aloe arborescens* and *Aloe variegata*. This investigation confirms the observation that the magnitude of diurnal fluctuations is influenced by cloudy sky, humid atmosphere, and supports the hypothesis that the rate of malic acid formation in succulent leaves when placed in dark is related to the intensity of