ORGANIC ACID METABOLISM OF THE BUCKWHEAT PLANT[1]

GEORGE W. PUCHER, ALFRED J. WAKEMAN, AND
HUBERT BRADFORD VICKERY

Introduction

Much of the recorded information concerning the metabolism of the organic acids in plant tissues has been obtained from measurements of diurnal changes either in the titratable acidity or in the hydrogen-ion activity of succulent plants. Such data yield little definite information with regard to the behavior of the individual acids present in the tissues. The calculation of the results of the titration of the organic acid in terms of grams or milliequivalents of any specific acid is usually based on the assumption that the predominating acid is the only one involved in the changes that may be observed. Although this is to some extent justifiable in many cases, the evidence is seldom satisfactory. BENNET-CLARK (**1**), in his careful review of the whole subject, points out that information on the intermediary metabolism of organic acids in plants is so fragmentary that "general pronouncements on the significance of the plant acids are at present probably of little value . . . and our knowledge of plant acid metabolism is therefore best summarized by including all the reactions which have been shown to occur in living tissues since it is usually impossible to show that such reactions do not occur in any given tissue."

Although the organic acid metabolism of plants other than succulents has been studied less extensively than that of the succulent plants, it is known that diurnal variations in pH occur in many species. Such changes have been interpreted by CLEVENGER (**2**), in the case of the cowpea, to signify an accumulation of organic acids during the night and utilization during the day. The changes he observed, however, are not nearly as pronounced as those often found in the Crassulaceae, and both GUSTAFSON (**3**) and HURD (**4**) have reported that the diurnal fluctuations in the pH of nonsucculents are not always associated with changes in the titratable acidity. SMALL (**16**) states that the variation in pH brought about by differences in the intensity of illumination of the plants is affected not only by the total concentration of acid but also by other factors connected with the metabolism of the living leaf, possibly the type of buffer system present, or the nature of the individual acids.

[1] The plants described in the present communication were grown at the New Jersey Agricultural Experiment Station. It is a pleasure to thank Dr. SHIVE and Dr. ROBBINS for the supply of seed, for their help and cooperation, and for the hospitality of the laboratory to one of us. A part of the expense of this investigation was borne by the Carnegie Institution of Washington.

The present study was undertaken to obtain preliminary data on the variations in the organic acids of a nonsucculent plant that shows diurnal periodicity in pH. Buckwheat (*Fagopyrum esculentum* Moench) was chosen since INGALLS and SHIVE (**5**) have shown that the pH of the sap expressed from both leaves and stems increases during a period of maximum illumination and decreases during the night.

Experimentation

The seeds were germinated between moist filter papers and were transferred to a germinating net as described by SHIVE (**13**). On April 4, 1937, when the seedlings were about 5 cm. tall, 72 plants were selected for uniformity of size and vigor, and were transferred, in groups of six, to 12 two-quart jars filled with a nutrient solution of the following composition: KH_2PO_4 0.00225 M, $Ca(NO_3)_2$ 0.0045 M, and $MgSO_4$ 0.00225 M. The nutrient solution also contained 0.5 to 1 part per million of iron and 0.25 parts per million each of boron and of manganese. The solution cultures were aërated continuously, and SHIVE's continuous-flow method of supplying fresh nutrient at the rate of about 500 to 700 ml. per day was employed. On April 28, the plants had attained a height of 55 to 60 cm. and were beginning to form flower buds. April 30, 26 days after transplantation, promised to be a bright and cloudless day so that maximal differences in light intensity during the sampling period seemed assured. Three cultures containing a total of 18 plants were therefore harvested at the start, and at each of three successive intervals during a 22-hour period. The plants were divided into leaf and stem tissue which were separately weighed and dried in a ventilated oven at 80° C. A 10-gm. aliquot of each fresh stem sample was frozen and the pH of the expressed sap was determined with a quinhydrone electrode. To conserve material for subsequent chemical analyses, pH determinations on the leaf tissue were omitted, since INGALLS and SHIVE have shown that the pH changes in the blade are similar in magnitude to those in the stem.

The four samples of leaf and stem tissue were obtained, one at 6:20 A.M., E.S.T., a second at 1:45 P.M., a third at 7:00 P.M., and a final one at 4:00 A.M. the following morning. The organic acid content of the dried samples was subsequently determined by specific methods (**6, 7, 8, 9**).

ORGANIC ACIDS OF BUCKWHEAT

Although extensive studies on the nutrient requirements of the buckwheat plant have been carried out by SHIVE (**14, 15**), there are, so far as we are aware, no data available on the organic acid composition of this plant. Table I shows the organic acid content of the initial sample of tissue in percentage of the dry weight. The chief acid of this plant is oxalic, the con-

TABLE I

ORGANIC ACID COMPOSITION OF BUCKWHEAT PLANT*

ACIDS DETERMINED	LEAF†	STEM†
	%	%
Oxalic acid	11.1	3.95
Malic acid	1.51	1.96
Citric acid	0.82	0.46
Nitrate N	0.37	2.07
Ash	13.27	17.48

* The average fresh weight of a single plant was 11.00 gm., of which 3.04 gm. were leaf, 7.19 gm. stem, and 0.765 gm. root. The average dry weight was 0.962 gm. distributed as 0.406 gm. leaf, 0.481 gm. stem, and 0.0753 gm. root.

† Data are given in percentage of the dry weight.

centration in the leaf being about three times as high as that in the stem. Malic and citric acid are also present in appreciable quantities, the former being slightly higher in the stem than in leaf tissue. Over 90 per cent. of the total organic acidity in the leaf is present as oxalic, malic, and citric acids, while in the stem only about 72 per cent. can be accounted for in terms of these three.

The stems of the plants contained a concentration of nitrate nitrogen more than five times greater than that of the leaf tissue. The quantity found is, in fact, the equivalent of nearly 15 per cent. of the dry weight, when expressed as potassium nitrate, and it is evident that a large part of the cations of the stem must be combined with nitrate ions. Accordingly a large proportion of the organic acids must be free or in the form of monobasic ions. This may account for the observation that buckwheat stem tissue, in spite of its greater ash and smaller organic acid content, is more acid in reaction than the leaf tissue.

Results

The behavior of the organic acids of buckwheat during the day may be most simply presented in terms of the changes in actual amount of each acid during each of the three successive time periods. These were selected to give first, an interval during which the illumination increased to a maximum and the reaction of the tissues, according to INGALLS and SHIVE, might be expected to become less acid; second, a period during which the light intensity diminished and the reaction of the tissues might be expected to become more acid; and last, a period of darkness when little or no change in pH occurs.

The changes are recorded in the tables as grams or milliequivalents, and also in percentage of the total amount of each substance present at the beginning of each successive period. Table II shows the changes in water, organic solids, and ash; table III shows the changes in organic acids.

TABLE II

CHANGES IN WATER, ORGANIC SOLIDS, AND ASH OF BUCKWHEAT PLANTS DURING THREE SUCCESSIVE TIME PERIODS IN ONE DAY

	LEAF*		STEM*		LEAF + STEM*	
	Period I, 6:20 A.M.–1:45 P.M.: increasing illumination and increase of pH of sap (4.20–4.40)					
	gm.	%	*gm.*	%	*gm.*	%
Water	+0.28	+10.63	+0.133	+ 1.98	+0.42	+ 4.50
Organic solids	+0.147	+41.7	+0.070	+17.6	+0.217	+29.0
Ash	+0.018	+33.4	+0.007	+ 8.33	+0.025	+18.1
	Period II, 1:45 P.M.–7:00 P.M.: decreasing illumination and decrease in pH of sap (4.40–4.24)					
Water	+0.197	+ 6.75	+0.914	+13.7	+1.11	+11.4
Organic solids	+0.025	+ 5.01	+0.038	+ 8.14	+0.064	+ 6.62
Ash	−0.002	− 2.78	−0.002	− 2.20	−0.004	− 2.45
	Period III, 7:00 P.M.–4:00 A.M.: complete darkness; no change in pH of sap (4.24–4.24)					
Water	+0.15	+ 4.83	+0.49	+ 6.32	+0.65	+ 5.98
Organic solids	−0.046	− 8.78	+0.113	+22.4	+0.06	+ 5.83
Ash	+0.004	+ 5.72	+0.015	+16.9	+0.019	+11.95

* The figures give the changes in grams per single plant in each period, and changes as percentage of the initial value in each period. The total amount of any constituent at the start of each period may be obtained by dividing the recorded quantitative change by the percentage change and multiplying by 100.

The first period is characterized by an increase in organic acids and, accordingly, the decrease in the acidity of the tissue extract cannot be interpreted as evidence for organic acid utilization. The observed increase of 0.2 unit in pH must have some other explanation such, for example, as an increase in absorption of inorganic cations, or the conversion of nitrate to ammonia. The increase of organic acid seems to be correlated with the photosynthetic processes in the plant since, from table II, it is apparent that the largest increase of organic solids, an approximate measure of photosynthetic activity, occurred during this period.

In the whole plant, 73 per cent. of the increase of total acids is due to oxalic acid and 27 per cent. to malic, citric, and unknown acids. Although the absolute amounts of acids formed, other than oxalic, are small, the percentage increases of these acids are large. The data also indicate that the individual acids are transported from leaf to stem tissue at markedly different rates. Thus malic acid increased 42 per cent. in the stem tissue and only 32 per cent. in the blade, while the unknown acids decreased in the

TABLE III

CHANGES IN THE ORGANIC ACIDS OF BUCKWHEAT PLANT DURING THREE SUCCESSIVE TIME PERIODS IN ONE DAY

	LEAF*		STEM*		LEAF + STEM*	
	Period I, 6:20 A.M.–1:45 P.M.: increasing illumination and increase of pH of sap (4.20–4.40)					
	meq.	%	*meq.*	%	*meq.*	%
Oxalic acid	+0.29	+28.7	+0.080	+19.3	+0.37	+25.9
Malic acid	+0.029	+32.2	+0.059	+41.9	+0.088	+38.1
Citric acid	+0.017	+34.0	+0.012	+34.3	+0.029	+33.3
Total gain of determined acids	+0.336	+29.2	+0.151	+25.6	+0.487	+27.9
Total organic acid	+0.38	+30.2	+0.13	+15.8	+0.51	+24.4
Unknown acids	+0.04	+36.4	−0.02	− 8.70	+0.02	+ 5.88
Nitrate N	+0.015	+14.1	+0.074	+10.4	+0.089	+10.9
	Period II, 1:45 P.M.–7:00 P.M.: decreasing illumination and decrease in pH of sap (4.40–4.24)					
	meq.	%	*meq.*	%	*meq.*	%
Oxalic acid	+0.04	+ 3.08	+0.017	+ 3.43	+0.05	+ 2.78
Malic acid	+0.002	+ 1.68	−0.030	−15.0	−0.028	− 8.78
Citric acid	+0.006	+ 8.70	−0.001	− 2.13	+0.005	+ 4.31
Total gain of determined acids	+0.048	+ 3.23	−0.014	− 1.89	+0.027	+ 1.20
Total organic acid	+0.13	+ 7.75	+0.055	+ 5.76	+0.18	+ 6.92
Unknown acids	+0.08	+53.3	+0.06	+28.6	+0.15	+41.7
Nitrate N	−0.020	−16.4	+0.042	+ 5.35	+0.022	+ 2.42
	Period III, 7:00 P.M.–4:00 A.M.: complete darkness; no change in pH of sap (4.24–4.24)					
	meq.	%	*meq.*	%	*meq.*	%
Oxalic acid	+0.14	+10.5	+0.058	+11.3	+0.20	+10.8
Malic acid	+0.019	+15.7	+0.097	+57.1	+0.116	+39.9
Citric acid	+0.016	+21.3	+0.013	+28.3	+0.029	+24.0
Total gain of determined acids	+0.175	+11.4	+0.168	+23.1	+0.345	+15.3
Total organic acid	+0.04	+22.6	+0.11	+10.9	+0.15	+ 5.40
Unknown acids	−0.13	−56.5	−0.06	−21.4	−0.19	−37.3
Nitrate N	−0.010	− 9.90	+0.085	+10.3	+0.071	+ 7.64

* The figures give the changes in grams per single plant in each period, and changes as percentage of the initial value in each period. The total amount of any constituent at the start of each period may be obtained by dividing the recorded quantitative change by the percentage change and multiplying by 100.

stem and increased 36 per cent. in the blade. These observations clearly show that data for the total organic acids alone cannot be used to interpret the metabolic changes in the individual acids. This has also been pointed out in the case of the organic acids of the rhubarb leaf (**12**).

In the second period, the increase of total organic acids in the leaf tissue was only one-third of that during the first period. Synthesis of malic, citric, and oxalic acid almost ceased, and 83 per cent. of the increase in total organic acids in the leaf and stem was due to the production of acids of the unknown group. This appears to be correlated with the fact that the plants received a diminishing amount of light and showed a corresponding diminution in photosynthetic activity. The definite loss of malic acid from the stem in this period shows that utilization of malic acid may occur when photosynthetic activity in the leaves is diminished. If the malic acid that disappeared had been transported from stem to leaf, there would have been a far more extensive increase in the leaf than was actually observed.

In spite of the utilization of malic acid in the stem, and the small increase in total acids, the tissue sap became more acid, a further indication that tissue pH is not a satisfactory index of the organic acid metabolism. Table II shows that no inorganic salt absorption occurred, and it therefore seems probable that the decrease in pH is associated with the establishment of a new equilibrium between the cations and the organic acids. The utilization of a part of the nitrate in the leaf suggests that some such rearrangement must have occurred.

The behavior of the organic acids during the period of darkness is particularly interesting. Table III shows that, although the total quantity of organic acids produced during the night by the leaf and stem was only 0.15 milliequivalent, the combined increase in oxalic, malic, and citric acid was 0.345 milliequivalent. This increase is clearly attributable to a transformation of acids of the unknown group, formed during the second period, into one or more of these three substances. In the second period, 0.15 milliequivalent of unknown acids were formed, in the third this fraction decreased by 0.19 milliequivalent. The agreement is well within the experimental error of these determinations. During the night, two distinct types of reaction appear to take place: a small quantity of organic acids is produced, and the unknown acids formed during the second period are converted into one or more of the known acids. The latter process recalls the observation that malic acid is converted into citric acid during culture of tobacco leaves in water in darkness (**11**). Table II shows that the buckwheat plant gains an appreciable quantity of ash during the night. It seems probable that a balance between cations and anions is thereby maintained so that no appreciable change in tissue pH occurs.

Discussion

It is obvious that, in the buckwheat plant, diurnal fluctuation in pH cannot safely be used alone as a criterion of the changes in the organic acid composition. It would seem that only a complete study of the variations in inorganic cations and anions, as well as of the organic bases and of the individual organic acids, would yield the information necessary to explain satisfactorily the observed fluctuations in reaction of the tissue juices. No such comprehensive set of data has, to our knowledge, ever been assembled for a plant tissue. In a recent paper (**10**), we have shown that a definite correlation exists, in the case of samples of tobacco leaf for which relatively complete ash analyses were available, between the excess of positive ions in the ash and the quantities of organic acids present in the tissue. The organic acids obviously play a major rôle in the physico-chemical relationships upon which the hydrogen-ion activity and buffer capacity of the cell fluids depend. The present results with buckwheat clearly indicate that these relationships are far from simple, and hold out little promise that they can be accounted for from such limited data as observations of the pH or of the titratable acidity.

Summary

The buckwheat plant contains oxalic acid as the chief organic acid of both leaf and stem. Malic acid is present in relatively smaller amounts and citric acid is present in minor proportions. These three acids make up about 90 per cent. of the organic acids of the leaf and about 72 per cent. of the organic acids of the stem. The leaves of one plant contain about 1.5 times as much total acid as the stem.

During the period of maximal illumination early in the day, photosynthesis is most active and the organic acids increase. Nevertheless there is a decrease in hydrogen-ion activity of the sap of the stem. In the later part of the day, malic acid is utilized and there is an increase in the group of unknown organic acids. The net change in total organic acids is a small increase, but the hydrogen-ion activity of the sap of the stem increases to approximately the early morning level. Photosynthesis is much less active in this period, and the assimilation of inorganic ions from the culture solution is also depressed.

During the night, there is a small increase in total organic acids and a marked interconversion of acids of the unknown group into one or more of the known acids takes place. There is no change in hydrogen-ion activity in the sap of the stem.

These observations show that interpretations of the changes in the hydrogen-ion activity of the sap of the plant in terms of an increase or decrease in organic acids cannot be made in the absence of specific information regarding these substances. It is obvious that such factors as absorption of inorganic ions by the roots, and the distribution of the inorganic and organic basic constituents within the tissues, as well as photosynthesis, play an important rôle in the changes in acidity that occur.

Connecticut Agricultural Experiment Station
New Haven, Connecticut

LITERATURE CITED

1. Bennet-Clark, T. A. The rôle of the organic acids in plant metabolism. New Phytol. **32**: 37–71, 128–161, 197–230. 1933.

2. CLEVENGER, C. B. Hydrogen-ion concentration of plant juices. II. Factors affecting the acidity or hydrogen-ion concentration of plant juices. Soil Sci. **8**: 227–242. 1919.
3. GUSTAFSON, F. G. Diurnal changes in the acidity of *Bryophyllum calycinum*. Jour. Gen. Physiol. **7**: 719–728. 1925.
4. HURD, ANNIE M. The course of acidity changes during the growth period of wheat with special reference to stem-rust resistance. Jour. Agr. Res. **27**: 725–735. 1924.
5. INGALLS, R. A., and SHIVE, J. W. Relation of H-ion concentration of tissue fluids to the distribution of iron in plants. Plant Physiol. **6**: 103–125. 1931.
6. PUCHER, G. W., VICKERY, H. B., and LEAVENWORTH, C. S. Determination of the acids of plant tissue. III. Determination of citric acid. Ind. & Eng. Chem., Anal. Ed. **6**: 190–192. 1934.
7. ————, ————, and ————. Determination of ammonia and of amide nitrogen in plant tissue. Ind. & Eng. Chem., Anal. Ed. **7**: 152–156. 1935.
8. ————, ————, and WAKEMAN, A. J. Determination of the acids of plant tissue. II. Total organic acids of tobacco leaf. Ind. & Eng. Chem., Anal. Ed. **6**: 140–143. 1934.
9. ————, ————, and ————. Determination of malic acid in plant tissue. Simultaneous determination of citric and malic acids. Ind. & Eng. Chem., Anal. Ed. **6**: 288–291. 1934.
10. ————, ————, and ————. Relationship of the organic acids of tobacco to the inorganic basic constituents. Plant Physiol. **13**: 621–630. 1938.
11. ————, WAKEMAN, A. J., and VICKERY, H. B. The metabolism of the organic acids of the tobacco leaf during culture. Jour. Biol. Chem. **119**: 523–534. 1937.
12. ————, ————, and ————. The organic acids of rhubarb (*Rheum hybridum*). III. The behavior of the organic acids during culture of excised leaves. Jour. Biol. Chem. **126**: 43–54. 1938.
13. SHIVE, J. W. A study of physiological balance in nutrient media. Physiol. Res. **1**: 327–397. 1915.
14. ————. A study of physiological balance for buckwheat grown in three-salt solutions. New Jersey Agr. Exp. Sta. Bull. 319. 1917.
15. ————. A comparative study of salt requirements for young and for mature buckwheat plants in sand cultures. Soil Sci. **6**: 1–32. 1918.
16. SMALL, J. Hydrogen ion concentration in plant cells and tissues. Protoplasma Monographien, zweiter Band. E. Steiger and Co. New York, 1929.