STUDIES IN CROP PHYSIOLOGY

Photosynthesis, Respiration and Pigment Content of Sugarcane Leaves in Relation to Age of Plant, Leaf Development and Nitrogen Deficiency

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Introduction

Earlier investigations showed that deficiency of nitrogen lowered the chlorophyll content of leaves (Singh, 1941; Lal, 1951). Nitrogen in doses below 33 per cent. of the total concentration of applied salts limited chlorophyll formation (Lal *et al.*, 1952). Its absence also caused normal leaf respiration at maturity (Lal *et al.*, 1951). Gregory and Richards (1929), Gregory and Baptiste (1936), and Gregory and Sen (1937) noted on the contrary, a subnormality in respiration of nitrogen-deficient barley leaves. It is intended in this paper to study the nitrogen deficiency effects in sugarcane leaves at various stages of the growth of the plant.

Experimentation

Sugarcane (Var. CoS. 109) was grown in sand nutrient cultures in Hoagland's complete nutrient and nitrogen-deficiency solutions as indicated in earlier paper (Lal and De, 1953 *a*). Samples of 6 leaves of various stages of development were taken at intervals of 45 days in the life-cycle. These leaves were picked up the previous evening and their rate of photosynthesis was determined on the following day under known conditions of light (500 watts Mazda lamp at a distance of 12 inches), temperature (30° C.) and CO_2 concentration (0·15 per cent.). Respiration of these leaves was also determined in darkness under otherwise similar conditions of temperature and CO_2. The details of the method have been described in earlier papers from this laboratory (Singh and Lal, 1935). Concentrations of chlorophyll *a*, chlorophyll *b*, carotin and xanthophyll in the leaf extracts were determined after Loomis and Shull (1937) using Guthrie's standard. Recorded observations in these directions were calculated to a known leaf surface or weight and statistically examined to indicate the response of plant age, leaf development and nitrogen deficiency on these plant characters.

Experimental Findings

Apparent Photosynthesis.—Rate of apparent assimilation, in mgm. of CO_2 per 100 sq. cm. leaf surface per hour, varied widely with plant age, leaf development and nitrogen deficiency. In general, highest

rates were recorded at 90 days and gradually declined with advance in age (Tables I–III). Apparent photosynthesis was also high in the first leaf from apex but declined as the leaf surface expanded. Nitrogen deficiency lowered the apparent assimilation rate at 45, 90 and 180 days but failed to indicate any marked effect at 135 and 225 days in the life-cycle.

Respiration.—Respiration in mgm. of CO_2 per 100 sq. cm. leaf area per hour showed the general tendency of high respiration during early stages and of low respiration at maturity. The decline with age was particularly evident beyond 135 days in complete nutrient plants and beyond 90 days under nitrogen deficiency. In fully manured plants, leaf respiration increased consistently from the first to the sixth leaf. Respiration of nitrogen-deficient leaves was highest in the second leaf from apex and showed a decline up to the fifth leaf. Nitrogen deficiency effects were more prominent in younger leaves where nitrogen deficiency showed tendency of higher respiration than the control. In the fourth to sixth leaves deficiency resulted in subnormality (Tables I–III).

TABLE I

Effect of plant age on the photosynthesis, respiration and pigment content of sugarcane leaves

(Over-all nutrition and leaf development values—Mean of 12)

	Age in days					S.D. at	
	45	90	135	180	225	1%	5%
Apparent photosynthesis ..	4·56	7·05	5·67	5·16	2·05	1·87	1·39
Real photosynthesis ..	7·42	10·18	8·35	7·16	3·20	2·30	1·71
Respiration ..	2·85	3·12	2·68	2·17	1·14	0·86	0·64
Chlorophyll *a* ..	5·77	8·22	6·02	3·24	1·21	1·45	1·08
Chlorophyll *b* ..	6·29	8·91	5·06	3·11	1·34	1·50	1·11
Carotin ..	4·90	9·07	6·89	5·43	3·01	1·42	1·05
Xanthophyll ..	7·15	10·28	10·95	7·59	3·48	1·43	1·06
Total green pigments ..	12·23	17·09	11·08	6·35	2·58	2·40	1·78
Total yellow pigments ..	12·05	21·01	17·84	13·02	6·49	2·56	1·91
Chlorophyll *a*/Chlorophyll *b*	0·96	0·92	1·27	1·11	0·94	0·34	0·25
Carotin ..	0·65	0·76	0·63	0·73	0·85	0·15	0·11
Total green/Total yellow ..	1·03	0·79	0·61	0·48	0·37	0·11	0·08

TABLE II

Effect of leaf development on the rate of photosynthesis, respiration and pigment content of sugarcane leaves

(Over-all nutrition and plant age values—Mean of 10)

	Leaf number from apex						S.D. at	
	1	2	3	4	5	6	1%	5%
Apparent photosynthesis ..	5·86	4·64	5·01	4·28	4·01	5·56	2·05	1·52
Real photosynthesis ..	8·03	7·07	7·39	6·68	6·09	8·28	2·52	1·87
Respiration ..	2·17	2·43	2·37	2·39	2·28	2·71	0·95	0·70
Chlorophyll *a* ..	3·93	5·13	4·96	5·13	5·77	4·47	1·59	1·18
Chlorophyll *b* ..	4·33	4·33	4·77	5·37	5·28	5·62	1·64	1·22
Carotin ..	4·83	5·77	6·11	6·29	6·01	6·13	1·56	1·16
Xanthophyll ..	7·06	7·74	7·81	9·23	9·07	8·43	1·57	1·17
Total green pigments ..	8·26	9·40	9·73	10·51	11·21	10·09	2·63	1·95
Total yellow pigments ..	11·89	13·51	13·91	15·52	15·09	14·56	2·81	2·09
Chlorophyll *a*/Chlorophyll *b* ..	0·97	1·21	1·13	0·93	1·16	0·83	0·38	0·28
Carotin/Xanthophyll ..	0·69	0·79	0·81	0·67	0·67	0·74	0·17	0·12
Total green/Total yellow ..	0·69	0·70	0·66	0·63	0·64	0·61	0·12	0·09

Real Photosynthesis.—Real photosynthesis was markedly reduced under nitrogen deficiency in all the successive leaves. Youngest leaf at the top and the sixth leaf from the apex showed highest rates when compared to leaves of intermediate insertion on the complete nutrient plants. Under nitrogen deficiency highest photosynthesis was recorded in the second leaf from apex. Real photosynthesis was also highest during early stages of growth and gradually declined towards maturity in both the cultures (Tables I–III).

Chlorophyll a.—The content of this pigment expressed in mgm. per 10 gm. fresh weight was highest in the leaf sampled at 90 days from fully manured plants and at 45 days in the samples from deficient cultures. In both, a gradual decline at later periods was evident. Nitrogen deficiency markedly reduced chlorophyll *a* between 90 and 180 days; and 45 and 225 days deficiency effects were less evident. Successive leaves from apex downwards also showed a fall in chlorophyll *a* content under nitrogen deficiency (Tables I–III*).

* Detailed tables from which the summarised results of Tables I–III were prepared, have not been given in text in view of space limitations.

TABLE III

Effect of nutrition on the rate of photosynthesis, respiration and pigment content of sugarcane leaves

(Over-all plant age and leaf development values—Mean of 30)

	Complete nutrition	Nitrogen deficiency	S.D. at	
			1%	5%
Apparent photosynthesis ..	8·48	4·32	1·19	0·88
Real photosynthesis ..	7·82	6·69	1·46	1·08
Respiration ..	2·41	2·38	0·55	0·41
Chlorophyll *a* ..	6·12	3·68	0·93	0·68
Chlorophyll *b* ..	5·93	3·96	0·95	0·71
Carotin ..	6·31	5·40	0·91	0·67
Xanthophyll ..	9·23	7·22	0·91	0·67
Total green pigments ..	12·09	7·64	1·52	1·13
Total yellow pigments ..	15·54	12·62	1·63	1·21
Chlorophyll *a*/Chlorophyll *b* ..	1·06	1·01	0·22	0·16
Carotin/Xanthophyll ..	0·70	0·76	0·09	0·07
Total green/Total yellow ..	0·72	0·59	0·07	0·05

Chlorophyll b.—Chlorophyll *b* content was highest at 90 days and fell with age in both the cultures. Nitrogen deficiency effects were less evident at 45 and 225 days. A marked reduction in chlorophyll *b* content between 90 and 180 days was noted in deficiency cultures. Nitrogen also reduced chlorophyll *b* content of successive leaves (Tables I–III).

Carotin.—Carotin content was highest at 90 days in sugarcane leaves and gradually declined with age irrespective of the nutritional conditions. At 90 and 135 days, nitrogen deficiency lowered carotin content but at later stages reductions were not so marked. With leaf development no marked variation in carotin was noted in either culture (Tables I–III).

Xanthophyll.—Xanthophyll content was also highest at 90 days and gradually declined towards maturity. Successive leaves from apex downwards were poorer in xanthophyll when nitrogen was withheld from the medium of growth. In complete nutrient plants xanthophyll content increased to a maximum in the fourth leaf and gradually declined in older leaves (Tables I–III).

Statistical analysis of the data showed the outstanding effect of nitrogen deficiency on apparent and real assimilation and the content of all the yellow and green pigments. Ratios between chlorophyll *a* and chlorophyll *b* and between carotin and xanthophyll were not significantly affected. The total green/total yellow pigment ratio, however, showed a marked response to conditions of nutrition. The effect of plant age on all the characters—respiration, photosynthesis, pigments and pigment ratios, was highly significant. Leaf age showed significant effect on total yellow pigments only. Interaction between plant age and nutrition was also significant on apparent assimilation, respiration, chlorophyll *a*, chlorophyll *b*, xanthophyll, total green and yellow pigments and the green/yellow ratio. Leaf age × nutrition interaction, on the other hand, failed to indicate any significant response on any of the characters. These indicated beyond doubt that the effect of nitrogen deficiency varied characteristically with age of the plant and less so with the development of leaves and their position of insertion on the main shoot (Table IV).

Effect of Plant Age

The over-all nitrogen deficiency and leaf development values showed a marked improvement in photosynthesis, respiration and pigment contents between 45 and 90 days and a significant fall at later stages. A higher rate of photosynthesis during early stages of the life-cycle

TABLE IV

Effect of age, leaf development and nitrogen deficiency on the rate of respiration, photosynthesis and pigment content of sugarcane leaves

Analysis of Variance

Due to	Degrees of Freedom	Mean sum of squares					
		Apparent assimilation	Real assimilation	Respiration	Chlorophyll *a*	Chlorophyll *b*	Carotin
Nutrition ..	1	20·10†	19·07†	0·01	89·18†	58·01†	12·37†
Plant age ..	4	40·64†	78·88†	7·30†	87·52†	101·95†	61·66†
Leaf age ..	5	5·24	6·75	0·33	4·00	3·01	2·81
Plant age × Nutrition	4	6·76*	7·82	2·12*	20·23†	10·52†	3·99
Leaf age × Nutrition ..	5	2·96	4·59	0·78	3·60	3·04	0·19
Error ..	40	2·79	4·23	0·60	1·71	1·80	1·62
Total ..	59	..	..	..	..	..	..
S.E./Observation ..	..	1·67	2·05	0·77	1·30	1·34	1·27

TABLE IV—(*Continued*)

Due to	Degrees of Freedom	Mean sum of squares					
		Xantho-phyll	Total green	Total yellow	Chl. *a*/ Chl. *b*	Carotin/ Xantho-phyll	Green/ Yellow
Nutrition ..	1	60·80†	297·26†	128·19†	0·04	0·05	0·27†
Plant age ..	4	136·23†	374·00†	375·54†	0·26*	0·08*	0·81†
Leaf age ..	5	7·06	10·14	16·91*	0·22	0·04	0·01
Plant age × Nutrition	4	10·29†	55·77†	26·22†	0·10	0·01	0·06†
Leaf age × Nutrition	5	1·12	9·58	1·70	0·22	0·03	0·04
Error ..	40	1·64	4·67	5·28	0·10	0·02	0·01
Total ..	59	..	..	..	..	..	..
S.E./Observation ..	..	1·28	2·14	2·29	0·31	0·14	0·10

* Significant effects at 5%. † Significant effects at 1%.

was associated with a higher rate of respiration and high pigment content of the foliage and the decline in photosynthetic rate at later periods was accompanied by a fall in respiration and pigment content (Table I). Comparative data on other plant processes (Lal and De, 1953 *a*, *b*, *c*, *d*) showed that high photosynthetic efficiency of the leaves between 45–90 days was also correlated with the highest accumulation of mineral contents and highest relative growth rate of the plant. At this period of high physiological efficiency of the plant, marked accumulation of insoluble nitrogen compounds also took place but sugars failed to accumulate beyond a certain extent. Lack of accumulation of sugars during early stages when photosynthetic rate of the leaves was at its highest involved two possibilities. In the first instance, they were rapidly broken down during respiration to provide the necessary energy for the various building up processes in the plant. Secondly, sugars formed were directly utilized in the synthesis of various nitrogenous compounds so essentially required for the formation of new tissues during the period of highest relative growth rate of the sugarcane plant.

High respiration during early stages of 45–90 days appeared to be mainly due to high fructose and reducing sugars and high concentration of amide and insoluble nitrogen fractions of the leaf. No specific relationship between amino acid concentration and high rate of respiration was evident as recorded by Gregory and Sen (1937) in barley. It appeared therefore, that hexoses, amides and proteins played a more important part in maintaining high respiration rate of sugarcane leaves during early stages.

It was also significant to note that the period of high chlorophyll content coincided with the period of highest photosynthetic activity and high magnesium, nitrogen and protein content of leaves. A fall in chlorophyll content towards maturity was associated with a fall in these ingredients. It was thus evident that maximum development of chlorophyll pigments was possible only when the leaf contained sufficient quantities of the three principal ingredients, *viz.*, nitrogen, magnesium and proteins.

Another significant point was the predominance of chlorophyll *a* over chlorophyll *b* content of leaves during early stages of the life-cycle. A marked decline in chlorophyll *a*/chlorophyll *b* ratio towards the later stages was indicative of a greater fall in chlorophyll *a* relative to chlorophyll *b* at maturity. Similarly, a marked decline in green/yellow pigment ratio indicated the predominance of yellow pigments over the green as age advanced. Both these contributed materially to the low photosynthetic activity of the sugarcane leaves at later stage of the life-cycle (Table I).

Effect of Leaf Development

Over-all nutrition and plant age values showed that apparent and real assimilation rates were more or less similar in leaves of various stages of development. Respiration also showed insignificant differences with leaf development. Barring carotin and xanthophyll which were richer in older leaves none of the pigments showed any marked fluctuation (Table II).

An inter-comparison of these effects with those recorded in earlier papers (Lal and De, 1953 *a*, *b*, *c*, *d*) showed that failure to exhibit marked fluctuation in photosynthesis, respiration and pigment content with leaf development could largely be attributed to the insignificant variation in the contents of glucose, fructose, amides, 'rest' and insoluble nitrogen fractions in the developing leaves. Although there was higher content of sucrose in the older leaves the respiration of such leaves decreased markedly. This indicated the possibility that sucrose was not an important respirable material in the sugarcane plant. Similarly, the insignificant effects of leaf development on plant pigments could be attributed to the insignificant variations in the content of protein and magnesium in similar leaves.

Effect of Nitrogen Deficiency

Over-all plant age and leaf development values indicated beyond doubt that nitrogen deficiency significantly reduced apparent and real photosynthesis (*cf.* Singh, 1941; Lal, 1951). Respiration of sugarcane leaves, on the contrary, was more or less normal under nitrogen deficiency (Table III). It appeared therefore, that the average response of nitrogen deficiency was normal on respiration and subnormal on photosynthesis of the leaves of sugarcane. In contrast to this, barley leaves showed subnormal respiration (Gregory and Sen, 1937) and normal photosynthesis (Gregory and Baptiste, 1936) when nitrogen was withheld from the medium of growth.

Data recorded in sugarcane indicated that subnormal photosynthesis was related to a significant decline in pigment content of deficient leaves. All the individual pigments were affected. Marked reduction in total green, total yellow and green/yellow ratio was recorded. In short, a predominance of yellow over green pigments under nitrogen deficiency conditions was associated with subnormal photosynthesis of sugarcane leaves. Further, the normal respiration of these leaves was found to be mainly due to two distinct effects which were discernible in these leaves (i) an improvement in glucose, fructose and reducing sugars, and (ii) a reduction in amino acids, amides and proteins under nitrogen deficiency conditions. If both these groups of compounds were capable of being utilized as respirable substrate, nitrogen deficiency should exhibit two distinct effects: (*a*) subnormal respiration due to lesser nitrogenous compounds, and (*b*) supernormality due to greater concentration of hexoses. Both these effects appear to proceed side by side but the increases in hexoses amply compensated the effects of decreases in active nitrogenous compounds with the result that respiration remained more or less normal. Supernormality in leaf respiration during early stages (90 days) under nitrogen deficiency appeared to be largely associated with higher content of hexoses and other sugars in deficient leaves rather than any marked improvement in nitrogen fractions. The subnormality in respiration at 135 days appeared related to a lowering in the concentration of both active sugars and organic nitrogenous compounds. Under conditions of nitrogen deficiency, therefore, leaf respiration was supernormal at 90 days, subnormal at 135 days and more or less normal at maturity. Normal respiration of sugarcane leaves at maturity under nitrogen deficiency conditions was also recorded in an earlier contribution (Lal *et al.*, 1951). A comparison of these effects showed that under the conditions of nutrition tried in these investigations hexoses played a more vital part than nitrogen fractions in the respiration of sugarcane leaves.

Summary

Under conditions of sand nutrient cultures a period of high photosynthesis, respiration and pigment content of sugarcane leaves was noted between 45 and 90 days. A significant fall in all these characters was observed between 180–225 days. High respiration during early stages was related to greater hexose, amide and protein content. Higher pigment concentration at this stage was largely due to high magnesium and protein content of leaves. A marked fluctuation in relative concentration of individual pigments was also recorded with a predominance of yellow over green pigments towards maturity.

With leaf development photosynthesis, respiration and pigment content showed no marked variation but carotenoids increased in older leaves. Nitrogen deficiency induced marked reduction in photosynthesis showing characteristic subnormal effects. Respiration was supernormal under similar conditions at 90 days and subnormal at 135 and 180 days with an average life-cycle value of respiration similar to that of the normal plants. In regulating respiratory activities of sugarcane leaves hexoses played a more vital part than the complex nitrogen compounds.

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