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Changes in photosynthetic carbon metabolism in senescent leaves of chickpea, *Cicer arietinum* L.

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Abstract

Photosynthetic processes in mature and senescent leaves of chickpea (*Cicer arietinum* L.) have been compared. With age, leaf photosynthetic pigments viz. chlorophyll a, chlorophyll b and carotenoids, and rate of 14 °C fixation were considerably affected. Analysis of $\delta^{13}C$, and short term photosynthetic products showed no major change in the path of photosynthetic carbon fixation. Study of long term photosynthetic ^{14}C assimilation revealed that in old senescent leaves, ^{14}C incorporation into organic acid and sugar fractions was enhanced.

Key words: chickpea, Cicer arietinum, photosynthesis, senescence

INTRODUCTION

In our previous paper we have reported alterations in inorganic constituents and enzyme activities in senescent leaves of chickpea (Murumkar and Chavan 1985). Chloroplasts represent a major site of protein storage (Huffaker and Peterson 1974) and the mobilisation of the corresponding nitrogen, along with the catabolism of chlorophyll in senescent leaves is highly significant with regard to the redistribution of nitrogen within plants. However, Hardwick (1983) considered the recycling of leaf nitrogen as adaptive in nitrogen-limited habitats, and the recycling of carbon as adaptive in habitats were the lower leaves get increasingly shaded. He has thus suggested that the seed legumes lose their leaves because the result of this process is an increase in fitness. Since the recycling of both carbon and

nitrogen would involve major alterations in the photosynthetic process, we thought it worthwhile to extend our studies to investigate the photosynthetic carbon metabolism in mature and senescent leaves of chickpea.

MATERIAL AND METHODS

The following photosynthetic parameters were studied on the mature and senescent leaves collected in a similar manner (Murumkar and Chavan 1985) from chickpea plants raised in sand culture in Hoagland nutrient medium:

1. $\delta^{13}C\{‰\}$. Dried leaf tissue was combusted in quartz tubes (6 mm O.D.) in a preheated 850°C oven for 1 h and allowed to cool slowly (40°C h^{-}). The tubes contained 10 mg of leaf tissue, 1.55 g of cupric oxide wire and 2 granulated copper. The CO_2 produced was analyzed on a Nuclide triple collector stable isotope mass spectrophotometer, after removing water vapour and non-condensable gases (e.g. N_2). The results were $\pm$ 0.1 ‰ standard error on the determination. The results expressed are relative to Pee Dee Belemnite (PDB).

2. **Photosynthetic studies.** For quantitative estimation of chlorophylls from acetone extracts, the method of Arnon (1949) was followed. Carotenoids were determined by reading absorbance of an acetone extract at 480 nm (Kirk and Allen 1965). The fresh leaf tissue was subjected to study of photosynthetic carbon assimilation following the method standardized in our laboratory (Hedge and Patil 1981). ^{14}C fixation studies were performed at 11.00 a.m. under optimum laboratory conditions (illuminance under HPLR lamp with intensity 0.6 Cal cm^{-2} min^{-1}; temp. 25 $\pm$ 2°C, humidity 60 $\pm$ 2%). The detached leaves were exposed to $^{14}CO_2$ by suspending on 50 mM Tris-HCl buffer pH 8.3 with $NaH^{14}CO_3$ (spec. act. 1.80 MBq mol^{-1}). The reactions were terminated after 5 seconds (short term) and 1 hour (long term) periods with boiling ethanol (80% v/v). The leaves were homogenized in ethanol and extracts were pooled and concentrated to a small volume. The insoluble residue was hydrolized with concentrated HCl and the neutral hydrolyzate was condensed in a similar manner. The products of short term (5 sec) and term (1h) ^{14}C assimilation were analyzed by two dimensional paper chromatography, cochromatography with authentic samples and autoradiography. The radioactivity in individual compounds was counted on the proportional counting system (EC model 2541, India).

Each experiment was performed in triplicate.

RESULTS

Photosynthetic pigments and rate of fixation of radiocarbon. Photosynthetic pigments viz. chlorophyll a, chlorophyll b and carotenoids were considerably lowered in senescent leaves. Chlorophyll b was destroyed more rapidly than

Table 1

Pigment composition, $\delta^{13}C$ values and photosynthetic ^{14}C fixation in mature and senescent leaves of chickpea, *C. arietinum* L.

Leaf	Photosynthetic pigments, mg 100 g^{-1} fresh tissue					$\delta^{13}C$ value, ‰	^{14}C fixation, Bq $min^{-1}mg^{-1}$ fresh tissue			Soluble: insoluble
	Chl a	Chl b	total Chl	Chl a:b	carote-noids		ethanol soluble fractions	ethanol insoluble fractions	total	
Mature	127.82	62.56	190.38	2.04	23.20	−29.0	15.41	11.25	26.66	1.37
Senescent	14.44	5.64	20.08	2.56	5.44	−28.6	2.17	0.16	2.33	13.56

Chl – chlorophyll. Data represents average of three determinations.

chlorophyll a, so the chlorophyll a:b ratio was elevated in older leaves. Advanced leaf age lowered the rate of photosynthetic fixation of radiocarbon; this decline was more rapid in the ethanol insoluble fraction and, as a result, the ratio of ethanol soluble to insoluble fraction was elevated considerably (about 10 fold) (Table 1). The value of $\delta\,^{13}C$ in senescent leaves was slightly less negative as compared to mature leaves.

Photosynthetic products. The distribution of radioactivity in products of short term (5 sec) ^{14}C assimilation in mature and senescent chickpea leaves is shown in Table 2. It is evident from the table that increased leaf age had not considerably altered the mode of photosynthetic ^{14}C fixation as no remarkable changes were observed in the labelling of C_3 (PGA, PEP, alanine, glycerate and glycine+serine) or C_4 (malate and aspartate) compounds. At the same time it is seen that in senescent leaves organic acid and sugar fractions received relatively more radioactivity.

The distribution of radioactivity in the ethanol soluble fraction during long term (1h) photosynthesis is recorded in Table 3. In older leaves, amino acids like alanine, glutamate, leucine and glutamine received less radiocarbon; other amino acids like cysteine, valine, isoleucine and proline accumulated more radioactivity than mature leaves (15 days). However, as a whole, the amino acid fraction labelling was lowered with increased leaf age. Incorporation of radioactive label into organic acids, malate, citrate and succinate decreased while that in photorespiratory compounds glycolate and glycerate was relatively higher in older senescent leaves than the mature leaves. Incorpora-

Table 2

Distribution of radioactivity among individual compounds* of ethanol soluble fraction following 5 sec ^{14}C photoassimilation by mature and senescent leaves of chickpea (*C. arietinum*)

Compound	Mature	Senescent
Total sugar phosphates and sucrose	**21.62**	**15.43**
SMP	2.71	T
PGA	3.19	8.74
PEP	6.77	6.69
Sucrose	8.95	**T**
Total organic acids	**16.29**	**29.48**
Malate	4.37	10.60
Citrate	4.45	6.82
Glycerate	4.22	7.42
Succinate	3.25	4.64
Total amino acids	**62.10**	**55.10**
Alanine	53.15	44.24
Aspartate	2.44	1.79
Glutamate	5.45	**T**
Glycine-Serine	1.06	9.07

T = Trace. * Values are expressed as percentage of total radioactivity counted on chromatogram and are mean of three determinations.

tion of ^{14}C in sugars such as sucrose and fructose increased in senescent leaves while that of glucose decreased. Older leaves had more radioactive label in PEP (phosphoenol pyruvate) while mature leaves had more PGA (3 phosphoglycerate).

The pattern of distribution of radiocarbon in the ethanol insoluble fractions is presented in Table 4. Though the distribution of radiocarbon in different amino acids was considerably changed, gross radioactivity in the amino acid fraction was higher in older leaves which had more ^{14}C

Table 3

Distribution of radioactivity among individual compounds* of ethanol soluble fraction following 1 hour ^{14}C photoassimilation by mature and senescent leaves of chickpea (*C. arietinum*)

Compound	Mature	Senescent
Total physophorylated compounds	**6.25**	**6.90**
PEP	2.04	6.40
PGA	2.45	0.25
SMP	0.11	T
UDPG	1.65	0.25
Total sugars	**20.19**	**25.52**
Sucrose	16.73	18.43
Glucose	1.33	0.49
Fructose	2.13	6.60
Total organic acids	**6.01**	**20.40**
Malate	8.57	4.11
Citrate	4.45	4.39
Isocitrate	0.89	3.58
Succinate	0.62	0.44
Ascorbate	0.45	0.86
Glycolate	0.24	0.92
Glycerate	0.43	1.59
Unidentified O.A.	0.36	4.51
Total amino acids	**57.55**	**47.18**
Alanine	28.33	17.49
Aspartate	7.88	8.22
Glutamate	13.92	5.59
Glycine-Serine	3.12	4.26
Cysteine	T	4.24
Valine	0.46	2.34
Phenylalanine	1.28	1.34
Leucine	1.15	0.93
Isoleucine	T	1.52
Tyrosine	0.53	0.14
Glutamine	0.69	0.52
Proline	0.19	0.41
Unidentified AA	T	0.18

T = Trace. * Values are expressed as percentage of total radioactivity counted on chromatogram and are mean of three determinations.

Table 4

Distribution of radioactivity* in ethanol insoluble fraction following 1 h ^{14}C photoassimilation in mature and senescent leaves of chickpea (*C. arietinum*)

Compound	Mature	Senescent
Glucose	9.10	5.80
Oxalate	0.38	0.78
Alanine	30.44	27.11
Glutamate	8.25	6.70
Aspartate	0.52	0.98
Glycine-serine	13.48	17.78
Cysteine	6.24	1.73
Cystine	0.33	T
Leucine	0.07	0.14
Valine	2.33	T
Proline	1.06	T
Methionine	0.77	10.65
Glutamine	6.19	7.06
Asparagine	2.33	0.93
Unidentified AA X_1	3.83	6.75
Unidentified AA X_2	2.66	1.63
Amino acids	78.50	81.46
Origin	12.00	11.96

T = Trace. * Values are expressed as percentage of total radioactivity counted on chromatogram and are mean of three determinations.

incorporation in amino acids like methionine, glycine, serine and glutamine. The level of radioactvity in oxalate was elevated while of glucose was decreased in senescent leaves.

DISCUSSION

The present findings clearly demonstrate that the chlorophyll content and rate of photosynthesis declined at nearly the same rate and these findings are in agreement with Secor et al. (1983), who have found a similar decline in chlorophylls and apparent photosynthesis in senescing soybean leaves. A decrease in carotenoids in senescent leaves supports the speculation of Sestak (1985) that the general trend of content of the sum of carotenoids during leaf ontogeny is similar to that of chlorophylls. The marked decline in both these pigment systems is obviously one of the main factors responsible for decline in photosynthetic efficiency of the senescent leaves.

$\delta^{13}C$ values stand for the mode of photosynthesis since the discrimination of $\delta^{13}C$ value is due to the efficiency of RuBP case enzyme to assimilate more ^{13}C than its other isotope; such a discrimination in CO_2 uptake is not shown by PEP case enzyme. According to Bender (1971) the $\delta^{13}C$ values of

-9 to -16 ‰ indicate the C_4 syndrome while $\delta^{13}C$ values of -23 to -32 ‰ indicate C_3 type of photosynthesis. Though slightly shifted towards less negative values, the $\delta^{13}C$ value of senescent leaves is clearly within the range of C_3 species, eliminating the probability of any major shift in the path of CO_2 fixation. Study of short term photosynthetic products (Table 2) supports this postulate since no major shift in incorporation of radioactive carbon from C_3 to C_4 compounds is noticed with advancement in leaf age. Though malate has received a high radioactive label in senescent leaves, it may be due to the increased respiration rate during senescence (Catsky et al. 1976) rather than any major shift in the mode of carbon fixation. The gross total of label in C_3 compounds (PEP, PGA, alanine, glycine and serine) is increased from 64.17 to 68.74%, on the other hand, the gross total of C_4 compounds (aspartate and malate) is increased slightly (6.81% to 12.39%) in senescent chickpea leaves and this fact overrules any such shift. In this respect chickpea leaf senescence differs from other plants like *Mollugo nudicaulis* where a shift from C_3 to C_4 was observed by Raghavendra et al. (1978) and *Portulaca oleracea*, a C_4 plant where a shift from malate to aspartate was reported by Kennedy and Laetsch (1973).

The alterations in activities of enzymes of the carbon reduction cycle during leaf ontogenesis ultimately lead to variations in the rate of synthesis of various compounds during steady state photosynthesis. The substantially raised ratio of soluble to insoluble ^{14}C fixation clearly sheds light on the fact that the major part of photosynthetically fixed carbon is shifted towards several soluble components rather than its incorporation towards synthesis of proteins and starch. Present findings of long term (1h) photosynthesis recall the work Joshi and Mishra (1970) who noticed enhancement of radioactive label in sugar and organic acid fractions at the expense of the amino acid fraction in senescent leaves of *Clerodendron inerme*. It is seen from Table 3 that incorporation of ^{14}C into the amino acid fraction is considerably lowered (an especially significant decline in alanine and glutamate) in old leaves indicating impairment of the protein synthesizing machinery during the senescent phase. At the same time, old senescent chickpea leaves show more incorporation of radiocarbon in sucrose and fructose along with PEP while the same in glucose is lowered. These observations indicate that the sugar metabolism is not much altered due to leaf senescence. The labelling of the organic acid fraction is more pronounced in old leaves since the radioactive label in isocitrate, glycolate, glycerate and ascrobate shows a definite increase. Thus, it is apparent that during leaf senescence, synthesis of some non-conventional (other than TCA cycle intermediates) organic acids is promoted. The present findings are in agreement with those of Raafat et al. (1971) who observed that total amounts of ^{14}C sugars and organic acids were highest in 21 day old primary leaves of *Phaseolus*, while the amount of ^{14}C amino acids was highest in 14 day old leaves. Our results have also shown that in photorespiratory products,

radiocarbon is consideraably accumulated in senescent leaves as more label in compounds like glycolate, glycerate, glycine and serine is evident.

Study of the ethanol insoluble fraction has revealed that at the senescent stage, incorporation in the amino acid fraction is considerably altered, since amino acids like methionine, glutamine, asparagine, glycine and serine have received more radioactive lable at the cost of alanine and glutamate, which are major amino acid pools in plants (Table 4). Senescent chickpea leaves also show more radiocarbon in oxalate. Mokronosov et al. (1973) observed that the amount of ^{14}C used for protein formation declined with leaf age. Hardwick et al. (1968) also observed a similar trend as the relative increase in ethanol--soluble photosynthates and a decline of ^{14}C in starch and proteins after 1h photosynthesis of 40 to 67 day old leaves of *Perilla frutescens*. It is evident from the foregoing account that although there seems to be no basic shift in the photosynthetic pathway due to senescence, the allocation of ^{14}C to various intermediates during long term photosynthetic carbon fixation in senescent chickpea leaves markedly differs from that in the mature leaves.

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Zmiany fotosyntetycznego metabolizmu węgla w starzejących się liściach ciecierzycy pospolitej, Cicer arietinum L.

Streszczenie

Porównano procesy fotosyntetyczne w dojrzałych i starzejących się liściach ciecierzycy pospolitej (*Cicer arietinum* L.). Wraz z wiekiem, w liściach wyraźnie zmieniały się barwniki fotosyntetyczne, tzn. chlorofil a, chlorofil b i karotenoidy oraz szybkość wiązania ^{14}C. Analiza $\delta^{13}C$ i produkty krótkotrwałej fotosyntezy nie wykazały zasadniczej zmiany w procesie fotosyntetycznego wiązania węgla. Badanie długotrwałej fotosyntetycznej asymilacji ^{14}C wykazało wzrost wbudowywania ^{14}C do kwasów organicznych i frakcji cukrowych w starych starzejących się liściach.